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CONTENTS

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EXPERIMENTS ON CHEMICAL CONTROL OF DAMPING-OFF IN *PINUS RESINOSA* AIT.[1]

BY L. P. V. JOHNSON[2] AND G. M. LINTON[3]

Abstract

Results from a series of greenhouse and nursery experiments on the relative control of damping-off in red pine, afforded by a wide range of chemicals, brought out the following points: (1) Semesan solutions in concentrations of 1 : 100 to 1 : 150 applied as soil treatment gave consistently the best control in both greenhouse and nursery. (2) Red copper oxide suspension in concentrations of 1 : 250 to 1 : 500 applied as a soil treatment was also effective, particularly in the greenhouse. (3) Red copper oxide and zinc oxide dusts used as seed treatments gave effective control under greenhouse conditions. (4) In the greenhouse, combinations of seed and soil treatments failed to prove more effective than treatments applied separately, while under certain nursery conditions the combined treatments were significantly less effective. (5) Fungicides as a group generally proved more effective than acidifying agents such as sulphuric acid and aluminium sulphate. It appeared that the acidifying agents gave good control in seasons of normal rainfall, but were more or less ineffective in dry seasons, when relatively large quantities of slightly alkaline irrigation water were applied, or in wet seasons, when excessive percolation occurred. (6) Seedlings from two-year-old red pine seed of somewhat reduced vitality proved to be much more susceptible than those from one-year-old seed.

White spruce was used in some of the preliminary experiments and proved to be much less susceptible than red pine, although very similar in response to the various treatments.

Introduction

Seedlings of several species of forest trees are subject to damping-off attacks prior to and for a few weeks after emergence from soil. Recurrent losses from this disease, particularly in red pine, coupled with an apparently inadequate existing knowledge of means of control, led the authors to undertake the greenhouse and nursery experiments reported herein.

The greenhouse studies, extending over the winters of 1939–40 and 1940–41 involved a series of experiments carried out in the following sequence: preliminary studies on chemical treatments, both seed and soil, conducted under different conditions of temperature and humidity, using both red pine and white spruce; exploratory tests with red pine in which 15 chemicals,

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mostly fungicides, were used at various concentrations and applied in different ways, singly and in combinations; and, combination seed and soil treatments, based on results of exploratory tests, tested on red pine in a factorial experiment.

A nursery experiment of factorial design involving combination seed and soil treatments based on greenhouse results was duplicated at Ottawa and Orono in 1941. Extensive experiments on soil acidification were also carried out at the Orono nursery during the period 1939–1942.

Because of the fact that each succeeding test was based on the preceding one, it has been considered sufficient to present details of only the later experiments. The results of most of the earlier work are discussed but not tabulated.

Literature reviews on the subject of damping-off control have been recently published by Boyce (1) and Horsfall (3).

Materials and General Methods

Red or Norway pine, *Pinus resinosa* Ait., was used throughout the tests; *Picea glauca* (Moench) Voss. was also used in some of the earlier experiments.

The chemicals tested included the fungicides, Semesan, Semesan Jr., mercuric chloride, ethyl mercuric bromide, red copper oxide, black copper oxide, copper carbonate, copper sulphate, zinc oxide, Du Bay, and Cheshunt compound, and the acidifying agents sulphuric acid, acetic acid, and aluminium sulphate. For the most part these chemicals have been recommended in the literature as being effective in reducing damping-off losses, especially in relation to vegetable garden plants.

The fungicide Semesan contains 30% hydroxymercurichlorophenol, the mercury equivalent in different lots used varying from 17.4 to 19.0%. The "New Improved" type of Semesan Jr. was used, which contains one per cent ethyl mercury phosphate. The preparation referred to as Du Bay is manufactured by the E. I. du Pont de Nemours and Co. Inc., Wilmington, Delaware, under the number 1155-HH. Cheshunt compound is a "home" preparation which contains 15.4% copper sulphate and 84.6% ammonium carbonate by weight.

The standard laboratory chemicals used were all of technical or commercial grade.

In the greenhouse experiments, water-tight crocks 6½ in. in diameter (inside) and 9 in. in height were used as soil containers. Each crock was brought up to equal tare weight and 4.5 kg. of soil of known moisture content added. This permitted maintenance of soil moisture content at approximately 20% by means of periodic weighing of the crocks and the addition of the amount of water necessary to bring them up to the required weight.

In steam sterilization, the soil was subjected to two hours treatment with live steam at 15 to 18 lb. pressure.

All tests were designed with either two or three randomized replicates.

Dosage for dust treatments was in all experiments the maximum amount adhering to the surface of dry seed.

Rates of seeding in the greenhouse varied with germinability of the seed used. The criterion was to sow the number of seeds which, on the basis of a prior germination test, would be expected to produce about 100 seedlings per crock. The white spruce seeds were stratified for five weeks at 36° F.

Greenhouse temperatures were controlled within one degree Fahrenheit—except during periods of particularly strong sunlight. Humidity was less accurately controlled by means of moist peat on and under the benches. Hydrothermographic records were kept during all experiments.

Seedling counts were made at the end of the fourth week after sowing and continued for five weeks thereafter. Total seedlings and number dampened-off were counted each week, the latter being removed to facilitate future counting. Records were kept in a cumulative manner. Only the data of the final count were used in the analyses.

Data on pre-emergence damping-off were obtained indirectly: instances where the total number of emerged seedlings in treatment crocks significantly exceeded the number in corresponding check crocks were taken to indicate a degree of control of pre-emergence damping-off.

Since the terms "pre-emergence damping-off" and "postemergence damping-off" are very inconvenient to use and since the latter is the one commonly dealt with, the term "damping-off" is used, unless otherwise noted, to refer to the postemergence form.

Experimental Results (Greenhouse)

Preliminary Experiments

1. *Relation of Temperature and Humidity to Damping-off*

Three greenhouse sections were maintained under more or less constant temperature and humidity conditions, as follows:

55° F. and 40 to 55% relative humidity,
70° F. and 25 to 35% relative humidity,
70° F. and 45 to 65% relative humidity.

The experiment involved a total of 216 crocks of contaminated soil sown in equal proportions to red pine and white spruce. Both seed and soil treatments were tested in replicate (the more pertinent results from which are included in Section 2 below).

At the lower temperature (55° F.), only a low percentage of damping-off occurred, but germination was very poor in red pine and only fair in white spruce.

At 70° F., the higher humidity (45 to 65%) significantly increased the number of seedlings emerged, and later significantly increased the percentage damping-off among emerged seedlings. The means by which the higher humidity produced increased emergence, whether as a beneficial effect on germination or as a reduction in pre-emergence damping-off, is not known. In any case, it was clear that the experimental conditions obtained at 70° F.

and the higher humidity were the most desirable for testing damping-off control, and hence were chosen for all subsequent greenhouse experiments.

2. *Tests of Seed and Soil Treatments*

The main preliminary experiment involved the testing of 15 chemicals which, in consequence of some being used as both seed and soil treatments or in more than one concentration, formed the basis of 41 different tests. Each test was replicated three times in Ottawa soil (inoculated with Orono soil) and three times in Orono soil (from beds heavily infested with damping-off organisms) making, with replicated steam sterilization treatment and checks, a total of 258 crocks. Only red pine seed was used.

The chemicals were applied as follows:

Liquid seed treatments—

Copper sulphate, 30 min. soaking in 1 : 80 solution.

Formalin, 60 min. soaking in 1 : 300 solution.

Dust seed treatments—

Semesan, Semesan Jr., red copper oxide, black copper oxide, zinc oxide, copper carbonate.

Liquid soil treatments—

Semesan (1 : 400, 1 : 200), Semesan Jr. (1 : 5000, 1 : 1000), sulphuric acid (1 : 200, 1 : 100, by volume), formalin (1 : 300, 1 : 150, by volume), aluminium sulphate (1 : 40, 1 : 20), mercuric chloride (1 : 400, 1 : 200), red copper oxide (1 : 500, 1 : 250), acetic acid (1 : 125), Cheshunt compound (1 : 100), ethyl mercuric bromide (1 : 100,000). Unless otherwise noted proportions are by weight. Dosage was 50 ml. (100 ml. in the case of acetic acid) per crock applied immediately after seeding.

Dust soil treatments—

Semesan (0.1), Semesan Jr. (0.1), black copper oxide (3.0), Du Bay (0.2, 0.3, 0.4, 3 days before seeding), zinc oxide (3.0), copper carbonate (3.0), aluminium sulphate (2.0). Numbers in parentheses denote amount in grams applied per crock.

Combination seed and soil treatments—

The dust soil treatments given above (with the exception of Du Bay and aluminium sulphate) were also applied to crocks sown with seed treated with the corresponding dust. A weekly treatment of 1 : 500 red copper oxide spray, 50 ml. per crock, was used alone and following red copper oxide seed treatment.

The experiment was first carried out using a two-year-old stock of red pine seed which, although germinating fairly well, produced seedlings of low vigour. The result was a very high pre-emergence mortality and practically complete postemergence mortality in the checks and in the more or less non-effective treatments in both soils. The results from the two soils will be discussed separately since analysis of variance gives a highly significant (1%) F value for the interaction between soils and treatments.

The disease was particularly severe in the Orono soil, where only five of the 123 crocks contained live seedlings at the end of the experiment. Two were from the Semesan (1 : 200) treatment, one each from mercuric chloride (1 : 400) and (1 : 200), and one from the red copper oxide weekly spray following seed treatment. The latter treatment gave the best control of pre-emergence damping-off, judging from emergence data. The red copper oxide, zinc oxide, and copper carbonate dust seed treatments also reached the 5% level of significance in this respect.

In the Ottawa soil pre-emergence damping-off was much less severe than in Orono soil, seedling emergence being about four times as great. Significant (5% level) reduction of pre-emergence damping-off was achieved by the following treatments: seed treatments involving red copper oxide, Semesan, zinc oxide, and copper carbonate; red copper oxide weekly spray following seed treatment; and combination seed and dust soil treatments involving Semesan, black copper oxide, zinc oxide, and copper carbonate.

Data on postemergence damping-off in Orono soil was not given statistical treatment because of the small numbers of emerged seedlings.

In Ottawa soil, significant (5% level) reduction of postemergence damping-off was afforded by the following soil treatments: Semesan (1 : 200), aluminium sulphate (1 : 40), ethyl mercuric bromide (1 : 100,000), and steam sterilization. Significant reduction was also obtained from the red copper oxide weekly spray following seed treatment.

The 18 liquid soil treatments of the above experiment—i.e., excluding seed treatments, dust soil treatments, and combination treatments—were repeated using a more vigorous stock of red pine seed. Again three replicates of each soil were used, the total number of crocks, including checks and a steam sterilization treatment, being 126.

Unlike the first experiment there was no significant difference between Orono and Ottawa soils with respect to treatment effect on seedling emergence, therefore results on emergence are averaged over the two soils. Two treatments, red copper oxide (1 : 500) and steam sterilization significantly increased emergence, while one, mercuric chloride (1 : 200) caused a significant decrease.

In the case of postemergence damping-off a highly significant (1% level) F value was obtained for the interaction, soils $\times$ treatments, hence results will be discussed separately for the two soils. In Orono soils, all treatments, except sulphuric acid (1 : 200), aluminium sulphate (1 : 40), acetic acid (1 : 125), and formalin (1 : 300 and 1 : 50) were effective. The first two are the weaker concentrations of acidifying agents, the higher concentrations of which were effective. In Ottawa soil, all treatments were effective with the exception of aluminium sulphate (1 : 40) and Cheshunt compound (1 : 100).

An interesting result from the repeated experiment is the relatively high resistance of vigorous seedlings (from fresh seed) to pre-emergence damping-off as compared with the weaker seedlings (from old seed) of the original experi-

ment. This is shown particularly by the emergence data from the highly polluted Orono soil. For example, in the original test using weak seed, 9, 20, and 61 seedlings respectively emerged from the three crocks of the treatment involving red copper oxide weekly spray following seed treatment (indicating a fair degree of germinability), while only 1, 0, and 1 seedling respectively emerged from the check crocks (indicating very little resistance to pre-emergence damping-off). In the repeated experiment, emergence from comparable treated crocks was 30, 27, and 20 seedlings, and from check crocks 30, 17, 32. This indicates that actual germination was probably no greater than in the original test, but that the more vigorous seedlings of the repeated test possessed a much greater resistance to pre-emergence damping-off.

Certain results from miscellaneous experiments should be mentioned. In tests of seed treatments using steam-sterilized and non-sterilized soil, treatment effects were completely obscured in the sterilized soil, indicating that damping-off organisms were not appreciably seed-borne. In another experiment, in which soil treatments were withheld until damping-off had commenced, it was shown that treatments that were definitely effective when applied at seeding time were practically ineffective when applied after damping-off had started.

3. *Effect of Soil Treatments on pH of the Soil*

The results from pH determinations made with a Beckman pH meter on the soil, following treatment, as well as on the treatment solutions themselves, are given in Table I. The effect of the acidifying agents, sulphuric acid and aluminium sulphate, are clearly marked, the former giving pH values averaging just above 3, while the latter gave values mainly in the range 3.5 to 4. Semesan proved to be the most alkaline treatment and gave slightly acid to slightly alkaline soil readings. All other treatments, and also the tap water check, gave slightly acid pH values.

TABLE I

pH DATA FROM DETERMINATIONS MADE IN CROCKS OF SOIL TREATMENTS APPLIED AT SEEDING AND REPEATED AT EMERGENCE (7 TO 12) AND OF SOIL TREATMENTS APPLIED AFTER DAMPING-OFF COMMENCED (13 TO 19)

Treatment chemical (pH in parentheses)	No. of crocks	Range of pH	Average pH
7. Mercuric chloride 6 : 100 (4.89)	12	5.45 – 6.32	5.86
8. Sulphuric acid 1 : 50 (0.47)	12	2.58 – 3.70	3.10
9. Aluminium sulphate 1 : 20 (3.18)	12	2.75 – 4.15	3.78
10. Formalin 1 : 150 (7.30)	12	6.10 – 6.86	6.49
11. Semesan 1 : 400 (11.42)	12	6.44 – 7.81	6.92
12. Tap water check (8.71)	12	6.27 – 6.97	6.60
13. Red copper oxide 1 : 500 (9.22)	4	6.00 – 6.60	6.39
14. Mercuric chloride, as above	4	6.07 – 6.39	6.23
15. Sulphuric acid, as above	4	3.35 – 3.72	3.56
16. Aluminium sulphate as above	4	4.20 – 4.99	4.65
17. Formalin, as above	4	6.10 – 6.43	6.26
18. Semesan, as above	4	6.09 – 7.25	6.49
19. Tap water check, as above	4	6.11 – 6.45	6.23

An attempt to correlate pH of the soil with damping-off control gave an insignificant r value.

Combination Seed and Soil Treatments

On the basis of previous results, a factorial experiment was designed in which 27 combinations of seed and soil treatments were tested in both Ottawa and Orono soil. The seed treatments were: red copper oxide, zinc oxide, copper carbonate, and untreated. Each was combined with each of the following soil treatments: untreated, Semesan solution (1 : 200 and 1 : 100), Semesan dust (0.5 gm. per crock), red copper oxide solution (1 : 250), red copper oxide dust (0.5 gm. per crock), and Du Bay dust (0.3 gm. per crock). A steam sterilization treatment, not part of the factorial experiment, was included since it is the common greenhouse procedure. All treatments were replicated twice in each soil, except the untreated and steam sterilized soil which were replicated three times. The experiment involved 126 crocks.

The method of applying treatments was as follows: 25 ml. of solution was applied to appropriate crocks immediately after sowing, repeated one week after sowing, and again (using 50 ml.) four weeks after sowing. Du Bay dust was applied immediately after sowing, other dusts one week after sowing. Dust treatments were not repeated.

The analysis of variance of the results is given in Table II.

TABLE II

Analysis of variance of results from combination seed and soil treatments (greenhouse)

Source of variance	Degrees of freedom	Mean square		
		Emergence	Damping-off	Survival
Between soils	1	3823**	2762**	151
Steam sterilization vs. all other treatments	1	2	5637**	1194*
Between seed treatments	3	453	1175**	261
Between soil treatments	6	3075**	4940**	2268**
Seed treatment × soil treatment	18	292	423	179
Soil × steam sterilization vs. all other treatments	1	84	213	317
Soil × seed treatments	3	408	10	282
Soil × soil treatments	6	592	377	176
Soil × seed treatment × soil treatment	18	327	247	176
Error	68	276	236	186

* *Exceeds mean square error, 5% level of significance.*

** *Exceeds mean square error, 1% level of significance.*

The significant difference between soils in relation to emergence and damping-off was due in both cases to consistently higher figures in the results from Orono soil. That this response to Orono soil was not differential with

respect to the various treatments is clearly shown by the complete absence of significant interaction between soils and treatments.

The steam sterilization data for damping-off and survival proved to be significantly better than corresponding data bulked for all other treatments. This significance is almost entirely due to the poor results from Du Bay dust. A fair appraisal of the other soil treatments in comparison with steam sterilization would be that red copper oxide was slightly inferior and Semesan slightly superior.

The significant difference in damping-off results between individual seed treatments resulted from red copper oxide and zinc oxide being distinctly better than copper carbonate in reducing damping-off. In the emergence data (pre-emergence damping-off control) each of the individual seed treatments gave considerably higher averages than the check.

The most significant point in Table II is the highly significant difference between soil treatments for each of the three kinds of data.

In Table III the data of all seed treatments and of both soils have been combined to illustrate the average effects of the various soil treatments on emergence, damping-off, and survival. It will be noted that all three of the Semesan treatments and the liquid red copper oxide treatment were significantly better than the check or the Du Bay treatment, and, except in the case of damping-off, than the red copper oxide dust treatment. Further note should be taken of the fact that Semesan 1 : 100 gave significantly better control of damping-off than any of the other effective treatments.

TABLE III

EFFECT OF SOIL TREATMENTS ON NUMBERS OF EMERGED AND SURVIVING SEEDLINGS AND ON PERCENTAGE POSTEMERGENCE DAMPING-OFF AVERAGED OVER ALL SEED TREATMENTS AND OTTAWA AND ORONO SOILS IN THE GREENHOUSE

Soil treatment	Treatment No.	Emergence	Damping-off	Survival
Check	1	37.8	92.3	2.3
Semesan 1 : 200	2	60.4*	63.1*	24.1*
Semesan 1 : 100	3	58.4*	48.2*	29.9*
Semesan dust	4	66.8*	63.3*	25.3*
Red copper oxide 1 : 250	5	54.9*	77.9*	12.1*
Red copper oxide dust	6	38.6	79.5*	10.0
Du Bay	7	32.1	94.5	1.9
Necessary difference (5% level)	1 and all others	10.7	9.9	8.8
	2 to 7	11.7	10.9	9.6

* *Significantly different from the check.*

Experimental Results (Nursery)

Combination Seed and Soil Treatments at Ottawa and Orono

Based on the results of the series of greenhouse experiments using Ottawa and Orono soils, a further experiment was designed to test some of the more promising soil and seed treatments, and combinations thereof, under practical conditions at the Ottawa and Orono nurseries in 1941. The seed treatments were red copper oxide, zinc oxide, and untreated, each being combined with each of the following soil treatments: untreated; red copper oxide (1 : 500 weekly and 1 : 250 bi-weekly); Semesan (1 : 300 weekly, 1 : 150 bi-weekly, and dust); aluminium sulphate (1 : 20 one application and 1 : 40 two applications). It will be noted that the differences in concentration for any chemical solution are compensated for by frequencies of application in such a way as to equalize the total amount of the chemical applied in any treatment. Each of the 24 treatments was applied, on a different randomization plan, to a plot in each of three beds at each nursery, making a total of 144 plots. Each plot, $2\frac{1}{2}$ sq. ft. in area, was sown immediately before treatment to 4.0 gm. of 1940 red pine seed. Seeds and chemicals used at both nurseries were from the same stock. The liquid soil treatments were applied at the rate of 250 ml. per sq. ft. at each application, and the dust soil treatment at the rate of 2.5 gm. per sq. ft.

Analyses of variance of the results from both the Ottawa and Orono experiments are given in Table IV. The effects on emergence differed widely between the two nurseries: at Ottawa the seed and soil treatments and interactions between them caused no significant differences, while at Orono highly significant effects resulted. The same is largely true for survival data. The effects of soil treatments on damping-off were significant at both nurseries.

TABLE IV

Analysis of variance of results from combination seed and soil treatments (Ottawa and Orono nurseries)

Source of variance	Degrees of freedom	Mean square					
		Emergence		Damping-off		Survival	
		Ottawa	Orono	Ottawa	Orono	Ottawa	Orono
Between replicates	2	4946	36719*	750	14	26118*	2628
Between seed treatments	2	7672	110875**	296	234	7671	38455**
Between soil treatments	7	28106	36024**	918**	1477*	18277*	32833**
Interaction, seed treatment × soil treatment	14	15641	23096**	269	182	11502	11628**
Error	46	14612	7279	238	109	7542	3297

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

Emergence, damping-off, and survival data (averaged over all seed treatments) in relation to the effect of soil treatment at both nurseries are given in Table V. Both of the Semesan liquid treatments, and red copper oxide (1 : 500 weekly) significantly reduced damping-off, and also significantly increased survival at Ottawa. At Orono, however, only Semesan (1 : 150 bi-weekly) resulted in significant reduction of damping-off. The unfavourable effect of certain soil treatments at Orono appears, from examination of the original data, to be due to a damaging effect resulting from the combining of different chemicals in applying soil treatments following seed treatments.

TABLE V

EFFECT OF SOIL TREATMENTS ON NUMBERS OF EMERGED AND SURVIVING SEEDLINGS AND ON PERCENTAGE POSTEMERGENCE DAMPING-OFF, AVERAGED OVER ALL SEED TREATMENTS, IN OTTAWA AND ORONO NURSERIES

Soil treatment	Emergence		Damping-off, %		Survival	
	Ottawa	Orono	Ottawa	Orono	Ottawa	Orono
Check	478	562	86.4	54.7	74	253
Red copper oxide 1 : 500 weekly	593*	515	71.6*	72.7†	164*	137†
Red copper oxide 1 : 250 bi-weekly	469	575	78.2	71.9†	110	158†
Semesan 1 : 300 weekly	421	403†	60.6*	53.0	172*	192†
Semesan 1 : 150 bi-weekly	470	486	59.0*	39.4*	195*	290
Semesan dust	508	489	76.4	78.9†	126	106†
Aluminium sulphate 1 : 20 one application	517	422†	76.9	64.2	130	165†
Aluminium sulphate 1 : 40 two applications	421	438†	84.9	62.0	70	180†
Necessary difference, 5% level of significance	115	81	14 6	9.9	82	54.0

* *Indicates significant favourable effect.*
† *Indicates significant unfavourable effect.*

The interaction between seed and soil treatments as it affects Orono results on emergence and survival is shown in Table VI. Except for one treatment, red copper oxide (1 : 500 weekly), the combination of seed and soil treatments substantially reduced emergence, even though seed or soil treatments alone almost invariably increased emergence. In the case of survival, the combination of seed and soil treatments was without exception highly unfavourable. Here again, the seed and soil treatments when used alone were almost invariably beneficial.

LARGE-SCALE ACIDIFICATION EXPERIMENTS AT ORONO

During the period 1939–1942 inclusive, extensive experiments on the effect of soil acidification on damping-off were carried out at the Orono nursery. Red pine was used in all experiments, the rate of sowing being 1 lb. per seed bed, size 4 × 30 ft. Treatment was applied immediately after sowing. Sulphuric acid was diluted at the rate of 22½ fl. oz. in 30 gal. of water. In 1939 and 1940 each bed was divided, half being treated, the other half left untreated as a check. In 1942 the beds were randomized in lines of five beds. Details of treatments and results are given in Table VII.

These results indicate that the severity of damping-off and the degree of control provided by acidification both vary widely in different years. In 1940, under conditions of very severe damping-off, good control was obtained

TABLE VI

INTERACTION BETWEEN SEED AND SOIL TREATMENTS WITH RESPECT TO EMERGENCE AND SURVIVAL AT THE ORONO NURSERY

Soil treatment	Number emerged (av. of 3 replicates)			Number surviving (av. of 3 replicates)		
	Seed treatment[1]			Seed treatment[2]		
	Check	Red copper oxide	Zinc oxide	Check	Red copper oxide	Zinc oxide
Check	507	587	591	188	300	272
Red copper oxide 1 : 500 weekly	502	503	540	156	150	105
Red copper oxide 1 : 250 bi-weekly	657	481	583	186	163	124
Semesan 1 : 300 weekly	478	355	375	263	168	143
Semesan 1 : 150 bi-weekly	541	455	441	373	250	246
Semesan dust	553	473	438	136	86	93
Aluminium sulphate 1 : 20 one application	524	267	474	219	73	204
Aluminium sulphate 1 : 40 two applications	683	238	393	322	55	163
Mean	556	421	480	231	156	169
Necessary difference	50			33		

[1] *Necessary difference, interaction, 140.*
[2] *Necessary difference, interaction, 94.*

TABLE VII

EFFECT OF ACIDIFICATION TREATMENTS ON DAMPING-OFF OF RED PINE SEEDLINGS AT THE ORONO NURSERY

Time of sowing	Precipitation 1 Apr.–30 June (in.)	Acidifier per bed	Treatment				Check			
			No. of beds	Average germination	Average survival	%	No. of beds	Average germination	Average survival	%
Fall, 1939	9.63	H_2SO_4 22½ fl. oz.	2½	11532	2874	24.9	2½	13536	680	5.0
Spring, 1940	9.36	H_2SO_4 22½ fl. oz.	2½	35340	11474	32.5	2½	34015	1793	5.3
Spring, 1941	3.40	H_2SO_4 22½ fl. oz.	65	13890	3850	27.7	56	14247	4047	28.4
Spring, 1942	12.76	H_2SO_4 22½ fl. oz.	10	27290	22911	84.0				
Spring, 1942	12.76	H_2SO_4 11¼ fl. oz.	10	24590	23010	93.6				
Spring, 1942	12.76	$Al_2(SO_4)_3$ 1 lb.	5	18820	15419	81.9	20	21918	17244	78.7
Spring, 1942	12.76	$Al_2(SO_4)_3$ 2 lb.	5	27980	23916	85.5				

in both fall- and spring-sown beds. In 1941, under drought conditions, damping-off was fairly severe, but acidification was completely ineffectual as a control measure. This might be explained on the basis of neutralizing effect of the irrigation water (pH about 8) which was applied by an overhead system. In 1942, under conditions of excessive natural precipitation, damping-off was not very severe, and acidification gave only slight to moderate increases in survival. Lack of better control in this case might be due to the removal of the acidifier by percolation. To go back to the 1940 results, it would seem that the good control provided by acidification might be explained by the assumption that, while the precipitation was sufficient to make irrigation with a more or less alkaline water unnecessary, it was not sufficient to render acidification ineffective through percolation.

General Discussion

The factorial experiments were designed primarily for the purpose of testing combination seed and soil treatments which, it was believed, might be superior to seed or soil treatments applied separately. However, upon combining some of the more effective treatments in the greenhouse and Ottawa nursery, it was found that prior seed treatments had no significant effect on results from superimposed soil treatments, while under nursery conditions at Orono, the combination treatments were significantly inferior to either form of separate treatment.

The most effective treatment proved consistently to be Semesan solution in concentrations of 1 : 100 to 1 : 150 applied to the soil at time of sowing and at emergence in the greenhouse, or at bi-weekly periods from sowing until four or five weeks after emergence in the nursery. Under greenhouse conditions, this treatment gave results equal to or slightly better than steam sterilization of the soil.

Next in efficiency was the soil treatment with red copper oxide suspension in concentrations of 1 : 250 to 1 : 500 applied according to the schedule given in the preceding paragraph. The use of this chemical as a seed and soil disinfectant has been intensively studied by Horsfall and co-workers (3).

A number of fungicidal dusts proved efficient as seed treatments, especially red copper oxide and zinc oxide used under greenhouse conditions. As a group, dust treatments proved greatly superior to liquid treatments as seed disinfectants.

The general results of the experiments also show that the fungicidal agents provided more consistent damping-off control than did acidifying agents. This is not in accord with the consensus of recent papers (2, 4, 5) in recommending acidification of the soil.

The fact that acidification gave little to no control in seasons of excessive rainfall or drought is presumed to be related to reduction of acidity to a non-effective level, by percolation in the one case and through application of slightly alkaline irrigation water in the other. Acidification apparently does not kill

the damping-off organisms, but prevents development by conditioning an unfavourable environment. Therefore, upon removal or neutralization of the acid, damping-off quickly develops. Fungicides on the other hand presumably kill the organisms and, therefore, provide a more positive control, less influenced by environmental conditions.

The disclosure in the present work of a strong indication that seedlings from two-year-old red pine seed of somewhat reduced vitality were far more susceptible to damping-off than those from fresh seed is considered to be a point of some importance. Since it is frequently necessary to store coniferous seed for two or three years owing to the spread of good seed years, this point brings up a storage problem that should not be disregarded.

Acknowledgments

The authors gratefully acknowledge the valuable assistance rendered by Dr. J. W. Hopkins and Staff in the statistical analysis of the data; by Messrs. E. C. Bradley, H. W. Holtz, L. Brown, and E. Emond in the experimental phases of the work; and by Dr. J. G. Malloch in preparing the manuscript.

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CONTRIBUTIONS TO A STUDY OF THE FUNGOUS FLORA OF NOVA SCOTIA

VI. PYRENOMYCETES[1]

By Lewis E. Wehmeyer[2]

Abstract

The 170 species and two varieties of fungi listed from the province are distributed in 70 genera as follows: Perisporiales—seven species and one variety in five genera; Hypocreales—29 species in 12 genera; Sphaeriales—129 species and one variety in 49 genera; Dothideales—five species in four genera. The following species are described as new: *Leptosphaeria anisomeres, Physalospora Laricis, Chaetosphaeria multiseptata, Massaria salilliformis, Cryptospora aurantiaca, Diaporthe quadruplex, Xylaria coprophila.* The following new combinations are made: *Bombardia (Sordaria) lutea* (E. & E.), *Pseudotrichia (Sphaeria) viridicoma* (Cke. and Pk.), *Pleospora (Teichospora) nitida* (E. & E.), *Apiognomonia (Gnomoniella) guttulata* (Starb.), *Gibberidea (Cucurbitaria) alnea* (Pk.).

The present paper is a report upon the pyrenomycetes collected over a series of summer field trips to the province of Nova Scotia. The reader is referred to the first paper of this series (20) for a description of the province in general and the localities mentioned, in particular.

These collections, again, represent only a small fraction of the species actually occurring in this region, for they were merely picked up in the course of general collecting. As very few pyrenomycetes have been reported from the province, however, they represent mostly new records.

The older arrangement of orders and families, as given by Winter (24), is followed. It is recognized that probably the bulk of the species placed in the Sphaeriales have a "perithecial" development considered to be pseudosphaeriaceous or dothideaceous. These terms are still largely theoretical, however, and apply to only a few species which have been studied in detail. There has never been any approach to an arrangement of the great bulk of existing species in the light of these theories and these terms are meaningless as taxonomic units for any thoroughgoing arrangement of species. The Allantosphaeriaceae and Diaporthaceae of Höhnel are recognized because they represent a distinct advance in the arrangement of the stromatic Sphaeriales and because the genera included have been assigned to these families. The few species of *Anthostoma, Fenestella, Valsaria*, etc. have been arbitrarily placed in the Diaporthaceae for want of a better repository.

Seaver's (16) arrangement of the Hypocreales has been followed, inasmuch as it is the only complete American account of this order.

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Contribution from the Department of Botany, University of Michigan, Ann Arbor, Mich., No. 818.

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Perisporiales

Capnodiaceae

Phaeocryptopus nudus (Pk.) Petr. (*Adelopus balsamicola* (Pk.) Theiss.). On killed needles of living trees of *Abies balsamea* (L.) Mill., Upper Brookside, June 27, 1931 (300*a*) and June 19, 1933 (1673).

Petrak (15) gives a full discussion of this genus and separates the fungus fruiting on the living needles of *Pseudotsuga* from this one on the killed needles of *Abies*.

Scorias spongiosa (Schw.) Fr. On living *Alnus* sp., Salmon River, July 14, 1931 (1018). Both the pycnidial and perithecial stages were found on this host.

Erysiphaceae

Erysiphe Galeopsidis DC. On *Chelone* sp., Upper Brookside, Aug. 28, 1931 (1420); Victoria Park, Sept. 5, 1935 (1682).

Erysiphe graminis DC. On *Agropyron repens* (L.) Beauv., Upper Brookside, June 29, 1931 (458).

Erysiphe Polygoni DC. On *Ranunculus acris* L., Upper Brookside, Aug. 28, 1931 (1423) and Aug. 31, 1931; on *Trifolium* sp., Onslow, Colchester Co., Aug. 31, 1931 (1497).

Microsphaera Alni (Wallr.) Salm. On *Alnus incana* (L.) Moench, Salmon River, Sept. 7, 1931 (1478); on *Syringa vulgaris* L., Upper Brookside, Sept. 11, 1931 (1494).

———— ———— var. **Vaccinii** (Schw.) Salm. On *Epigaea repens* L., Grande Anse, Richmond Co., Aug. 3, 1931 (1238).

Phyllactinia corylea (Pers.) Karst. On *Alnus incana* (L.) Moench, Salmon River, Sept. 7, 1931 (1478).

Hypocreales

Nectriaceae

Letendraea luteola E. and E. On decayed wood, Salmon River, July 14, 1931 (1026).

Nectria coccinea (Pers.) Fr. On living bark and on cordwood of *Fagus grandifolia* Ehrh., Truro, July 11, 1929 (37); Salmon River, July 15, 1931 (1041).

This is the species of *Nectria* associated with the beech bark disease in the northeast. Ehrlich states that it differs from Seaver's (16, p. 21) description of *Creonectria coccinea* in the smaller spores, but is probably a form of *Nectria coccinea*.

Nectria (*Creonectria*) **Coryli** Fck. On *Salix* sp., Upper Brookside, July 13, 1931 (1003).

Nectria (*Creonectria*) spp.

The stroma-forming species of *Nectria* of North America are so poorly known and their nomenclature so confused, that it has been impossible to place several collections. The description of these collections is given here in the hope that they may be properly placed at a later date.

A—On *Betula* spp., Economy Lake, June 16, 1926 (*P73a*); Victoria Park, Truro, Aug. 15, 1933 (1631).

Perithecia bright, then dull red, 300–350 μ in diameter, crowded upon an orange-red, erumpent stroma, spheric at first, smooth, becoming collapsed. Ascospores variable in shape and size, cylindric to ellipsoid, straight or slightly curved, usually constricted, two-celled at first, in age becoming three- to four-celled, (13) 15–22 $\times$ 4–7 μ. In both these collections the ascospores were accompanied by numerous cylindric to allantoid, hyaline, one-celled conidia, 5–7 $\times$ 1.5–2 μ. These may be sprout conidia from the ascospores, but no actual attachment was seen. In No. 1631, long, lunate, *Fusarium*-like conidia were seen. These were 40–63 $\times$ 2.5 μ.

B—On *Acer* sp., Salmon River, Oct. 2, 1926 (96), leg. A. R. Prince.

Perithecia pale red to orange-red, 300 μ in diameter, thickly clustered on a cream to yellowish stroma, very slightly roughened, with an umbilicate ostiole, finally somewhat collapsed. Asci 53–63 $\times$ 8.5–10 μ. Spores mostly long-cylindric, straight or slightly curved, very slightly or not at all constricted, two-celled, septum faint, hyaline, 12.5–14.5 $\times$ 3.5–4.5 μ.

C—On *Betula papyrifera* Marsh., Upper Brookside, July 13, 1931 (1010).

Perithecia bright red becoming deep blood red, 200–250 μ in diameter, with a prominent ring-like thickening about the upper portion, through which the ostiole is erumpent. Asci cylindric 50–60 × 3.5 μ. Spores uniseriate, ellipsoid, hyaline, two-celled with a very faint septum, ends rounded, 7–9 × 2.5–3.5 μ.

These collections were submitted to Dr. Seaver for an opinion. He suggests that *A* and *B* may be *Creonectria purpurea* (L.) Seav. The spores are of a size found in this species, but the perithecia do not show the coarse roughness characteristic of it. *C* he places as *Nectria pithoides* E. and E., which is also the writer's opinion. Wollenweber (25, p. 185) gives this species as a synonym of *N. applanata* Fck.

Nectria episphaeria (Tode) Fr. On sphaeriaceous stromata on *Betula*, Wolfville, June 26, 1926 (*P25a*); Upper Brookside, July 31, 1931 (386*a*); on *Corylus cornuta* Marsh., Earltown Rd., Aug. 22, 1931 (308*b*); on *Dermatea* on *Abies*, Pictou Rd., June 30, 1931 (530).

Common on stromata of various fungi particularly the Sphaeriales.

Nectria lactea Ell. and Morgan. On myxomycete sporangia and on wood upon which they are growing, Upper Brookside, July 17, 1931 (1071 and 1071*a*), leg. A. H. Smith.

The perithecia are minute, white, and covered with a cottony tomentum, giving them the appearance, superficially, of a parasitic hyphomycete.

Nectria Peziza (Tode) Fr. On *Polystictus* sp., Upper Brookside, July 24, 1929 (100); on decayed wood, Upper Brookside, July 16, 1931 (1064 and 1065).

Nectria sanguinea (Bolt.) Fr. On conifer stump, Earltown Rd., Aug. 26, 1931 (1411).

This collection is placed here on account of its occurrence on decorticated wood. It is scarcely distinguishable from *N. episphaeria*. Seaver distinguishes *N. sanguinea* from *N. episphaeria* by the broad-fusoid (10–12 × 4–5 μ) instead of narrow-fusoid (9–12 × 4–6 μ) spores, but there is obviously very little difference.

Passerinula candida Sacc. Parasitic on stromata of some discomycete on *Abies balsamea*, New Glasgow Rd., July 25, 1931 (1148).

Perithecia immersed in the disk-shaped, black, leathery, partially erumpent stromata of a *Dermatea* or *Tympanis*, 200–250 μ in diameter, walls reddish, parenchymatous, appearing like those of a *Nectria*, erumpent as small, papillate, reddish-black ostioles. Asci cylindric, 90–130 × 10–11 μ. Spores uniseriate to irregularly biseriate, ellipsoid to fusoid-ellipsoid, ends blunt, two-celled, granular, hyaline at first, becoming brownish, (14) 17–21 × 8–9 μ.

Passerinula candida, which is reported as parasitic on pyrenomycete stromata, has not been seen. The description of that species differs only in the different ostioles, smaller perithecia ($\frac{1}{6}$–$\frac{1}{8}$ mm.), the attenuate–stipitate asci and the four-guttulate spores. Whether or not these are varietal or specific differences can be determined only by comparison with authentic material of this species.

Scoleconectria balsamea (Cke. and Pk.) Seav. On *Abies balsamea*, New Glasgow Rd., July 25, 1931 (1147 and 1148).

The spores of these collections measure 14–28 × 3.5–5 μ.

Scoleconectria scolecospora (Bref.) Seav. On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929 (29); Earltown Rd., Aug. 22, 1931 (362*a*).

The spores of this species are 30–60 × 2–3 μ. Both this and the preceding species show numerous sprout conidia in the asci, and are fairly common on fir.

Hypocreaceae

Byssonectria violacea (J. C. Schm.) Seav. On *Fuligo* sp., Aug. 15, 1931 (1323).

Chromocrea gelatinosa (Tode) Seav. On *Alnus* sp., Aug. 14, 1935 (1680).

The stromata are flat-pulvinate, cream-yellow at first, becoming greenish because of the exuded spores which are dark green to green-brown at maturity.

Claviceps purpurea (Fr.) Tul. On *Agropyron repens*, Onslow, Colchester Co., Aug. 31, 1931 (1440), sclerotia only.

Cordyceps capitata Link. On *Elaphomyces* sp., Wentworth Valley, Cumberland Co., Aug. 29, 1931 (1433), leg. A. H. Smith.

Cordyceps militaris (L.) Link. On pupa, Lake O'Law, Inverness Co., Aug. 6, 1931 (1070*a*); on cocoons, Upper Brookside, Aug. 16, 1927, leg. A. R. Prince (6217); July 17, 1931 (1070), leg. A. H. Smith.

Cordyceps ophioglossoides (Ehr.) Sacc. On *Elaphomyces* sp., Northeast Margaree, Sept. 4, 1927 (388).

Cordyceps stylophora Berk. and Br. On beetle larvae, Upper Brookside, July 25, 1931 (1167), det. E. B. Mains.

Cordyceps viperina Mains. On beetle larvae, Upper Brookside, July 25, 1931 (1213); Earltown Rd., Aug. 26, 1931 (1213*a*).

These are two of the collections upon the basis of which Mains (11) described this species.

Hypocrea citrina (Pers.) Fr. On *Fomes pinicola* (Swartz) Cke., Upper Brookside, July 4, 1931 (386); on *Fomes applanatus* (Pers.) Gill, Princeport, Colchester Co., July 9, 1931 (468).

Hypocrea patella Cke. and Pk. On decayed beech log, Folleigh Lake, July 20, 1931 (1094). The stromata vary from almost white to bright yellow or ochraceous.

Hypocrea rufa (Pers.) Fr. On *Fagus grandifolia*, Upper Brookside, July 11, 1931 (494); on *Alnus* sp., Salmon River, July 15, 1931 (494*a*).

Quite common on the moist surface of down logs.

Hypomyces apiculatus (Pk.) Seav. On humus of old stump, Salmon River, Sept. 7, 1931 (1475).

Hypomyces aurantius (Pers.) Tul. On *Irpex* sp. on birch, Upper Brookside, July 24, 1929 (99); on *Boletus*, Killag Mines, July 31, 1931 (1178); on *Polystictus versicolor* (L.) Sacc., Upper Brookside, Aug. 24, 1931 (1397).

Hypomyces chrysospermus (Bull.) Tul. On an agaric, Salmon River, Aug. 18, 1931 (1350); on *Boletus*, Folleigh Lake, Aug. 29, 1931 (1455), leg. A. H. Smith.

Sydow (18, p. 186) described the genus *Apiocrea* for those species of *Hypomyces* whose spores have two cells of unequal size and cited the species *Hypomyces chrysospermus* (Pers.) Tul., *H. hyalinus* (Schw.) Tul., and *H. Tulasneanus* Plowr. as belonging in this genus. Seaver (16) gives *H. chrysospermus* as having spores slightly roughened at maturity and 12–15 × 4 μ. European descriptions of this latter species give its spores as 21–30 × 5–6 μ. The spores of *H. Tulasneanus* are given as 20–25 × 8 μ. These two collections from Nova Scotia have the same appearance, but the ascospores of No. 1350 are large, 17.5–21 × 4–5 μ, have a long appendage at each end, and are smooth and somewhat constricted at the septum. The conidia of the *Sepedonium* stage are comparatively short-tuberculate. This appears to be the European *H. chrysospermus*. No. 1455 has shorter spores, 9–12 × 2.5–3.5 μ, with shorter appendages, and the conidia have longer, cylindric tubercules. This may be the *H. chrysospermus* of Seaver.

Hypomyces lactifluorum Schw. On an agaric, Upper Brookside, Aug. 31, 1925, leg. A. R. Prince (1099); quite common.

Hypomyces polyporinus Pk. On *Polystictus versicolor*, Earltown Rd., Aug. 19, 1931 (1368).

Hypomyces rosellus (Alb. and Schw.) Tul. On decayed *Poria*, Upper Brookside, Aug. 24, 1931 (1396).

Peckiella viridis (Alb. and Schw.) Sacc. On *Russula* spp., Upper Brookside, July 16, 1931 (1058); Sept. 4, 1931, leg. A. H. Smith.

Podostroma alutaceum (Pers.) Atk. On moss and duff under conifers, Salmon River, Aug. 13, 1931 (1293), leg. A. H. Smith; Aug. 18, 1931 (1293*a*).

Sphaeriales

Chaetomiaceae

Chaetomium globosum Kze. In gross culture of dung (grouse?), Upper Brookside, 1931.

Although the tips of the hairs were more spirally coiled than described by Chivers (4, Pl. 15, Fig. 11) for *C. globosum*, two types of hairs as in *C. cochlioides* could not be distinguished.

Chaetomium indicum Corda. In gross culture of deer dung, Upper Brookside, (*C2*) det. by Ralph Bennett.

Sordariaceae

Bombardia coprophila (Fr.) Kirschst. On cow dung, Northeast Margaree, Sept. 4, 1927 (*P*434); Portapique Beach, July 26, 1933 (1595); on porcupine dung, Mt. Thom, Aug. 15, 1931 (1313).

Bombardia lutea (E. and E.) comb. nov. (*Sordaria lutea* E. and E., J. Mycol. 3 : 118. 1887). On decayed wood, Killag Mines, July 30, 1931 (1187); Upper Brookside, July 31, 1931 (1187*a*).

As has been pointed out by Cain (2, p. 66), this species has the asci of the genus *Bombardia*. They are long-cylindric, tapered toward the apex and have a cylindric plug in the tip. It is transferred to *Bombardia* for this reason. Ellis (5, p. 132) gives the asci as 12- to 16-spored and transfers the species to the genus *Philocopra* on this basis. These collections appear to have eight-spored asci. Ellis also fails to mention or figure the secondary appendages, which were seen on these spores, and which appear at each end, and are filiform, 15–20 μ long. The perithecia are not stromatic in this fungus, unless the outer layer of yellowish tomentum of the thick-walled perithecia be considered as a stroma.

Sordaria appendiculata Auersw. On rabbit dung, Green Oaks, Colchester Co., July 12, 1929 (178); New Glasgow Rd., July 25, 1931 (1145); Upper Brookside, July 27, 1931 (1145*a*); on porcupine dung, Upper Brookside, Aug., 1931 (1492).

Sordaria fimicola (Rob.) Ces. and de Not. On moose dung, Killag Mines, July 30, 1931, det. Ralph Bennett.

Sporormia ambigua Niessl. On horse dung, Earltown Rd., Aug. 22, 1931 (1393).

Trichosphaeriaceae

Apiosporina Collinsii (Schw.) Höhn. Causing a witches' broom on *Amelanchier* sp., Bass River, July 26, 1935 (1752).

Chaetosphaeria multiseptata sp. nov. (Figs. 1, 2).

Perithecia superficial, gregarious to crowded, spheric, 250–300 μ in diameter, black, carbonous, brittle, sparsely covered with short, stiff, dark brown, septate hairs, 30–70 $\times$ 5–6 μ. Subiculum lacking or very sparse. Ostiole short, conic-papillate. Asci clavate with a refractive ring in the slightly thickened apical wall, 90–125 $\times$ 10.5–12.5 μ. Paraphyses filiform, hyaline, persistent. Spores biseriate, oblong-fusoid to cylindric, straight or somewhat curved, brown, six- to eight-celled with the end cells small, cap-like and hyaline, not constricted at the septa, 32–39 $\times$ 5–6 μ, with a large guttule in each cell.

Type: Herbarium L. E. Wehmeyer, on decayed stump, Upper Brookside, Colchester Co., N.S., 26, VII, 1929, leg. L. E. Wehmeyer (No. 78).

Although the perithecia are very sparsely setose and without a subiculum, the spores of this species are typical of *Chaetosphaeria*. The only closely related species are *C. Rehmiana* (Henn.) Kirschst. and *C. caelestina* Höhn, both of which are larger throughout, are described as having six-celled spores, and occur on tropical hosts.

Chaetosphaeria multiseptata sp. nov. Perithecia superficialia, gregaria vel dense aggregata, sphaerica, 250–300 μ diametro, nigra, carbonea, sparse pilis rigidis atrobrunneis septatis 30–70 μ longis, 5–6 μ diametro vestita. Subiculum nullum vel sparsum. Ostiolum breve, conice papilliforme. Asci clavati, 90–125 μ longi, 10.5–12.5 μ crassi, apice annulo refractivo praediti et membrana paulum incrassata. Paraphyses filiformes, hyalinae, persistentes. Sporae biseriatae oblonge fusiformes vel cylindricae, rectae vel leviter curvatae, brunneae, 6- vel 8-cellulae, ad septa non constrictae, 32–39 μ longae, 5–6 μ crassae, cellulis uniguttulatis, terminalibus hyalinis, parvis, hemisphaericis.

Specimen typicum legit L. E. Wehmeyer, No. 78, in ligno putrido prope "Upper Brookside, Colchester Co." in Nova Scotia, 26, VII, 1929.

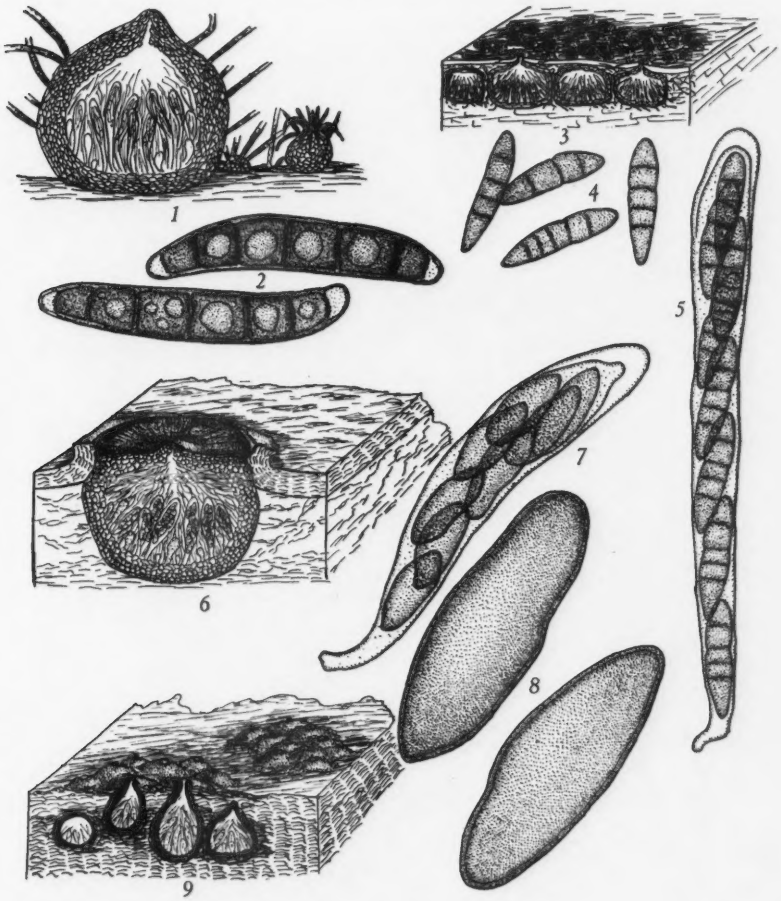
Species subiculo nullo etiamque sparsitate setorum aberrans sed sporis *Chaetosphaeriae* typicis, affinis est *C. Rehmianae* et *C. caelestinae*, speciebus tropicalibus omnino grandioribus, cum sporis 6-cellulis.

Helminthosphaeria Clavariae (Tul.) Fck. Parasitic on *Clavaria cristata* Holmsk. ex Fr., Upper Brookside, July 27 and Aug. 27, 1931 (1164 and 1669); on *Clavaria cinerea* Bull., Upper Brookside, Aug. 20, 1931 (1374) leg. A. H. Smith.

The dark brown hyphae of the parasite can be seen penetrating throughout the tissue of the host, and cause a deformation and blackening of the stalks. At the surface these parasitic hyphae protrude and bear the conidial stage, *Scolecotrichum Clavariarum* (Desm.) Sacc.

Herpotrichia pezizula (B. and C.) E. and E. On *Acer spicatum* Gam., Upper Brookside, Aug. 12, 1931 (1288).

This is accompanied by the conidial stage, *Helicoma Curtisii* Berk.



FIGS. 1 AND 2. *Chaetosphaeria multiseptata Wehm.*

FIG. 1. *Vertical section of perithecium and perithecial primordia.* FIG. 2. *Ascospores.*

FIGS. 3 TO 5. *Leptosphaeria anisomeres Wehm.*

FIG. 3. *Radial and surface views of perithecia.* FIG. 4. *Ascospores showing irregular septation.* FIG. 5. *Ascus.*

FIGS. 6 TO 8. *Physalospora Laricis Wehm.*

FIG. 6. *Radial section and surface view of perithecium on Larix.* FIG. 7. *Ascus.* FIG. 8. *Ascospores.*

FIG. 9. *Radial section and surface view of perithecial clusters of Massaria salilliformis Wehm.*

Lasiophaeria hirsuta (Fr.) Ces. and de Not. On decayed log, Mt. Thom, Aug. 10, 1931 (1253).

The spores of this material are one-celled, hyaline at first, becoming pale brown and four-guttulate, 55–59 × 4–5 μ. The one-celled condition of the spores suggests *L. strigosa* (Alb. and Schw.) Sacc. but the spores are too long for that species. The septa

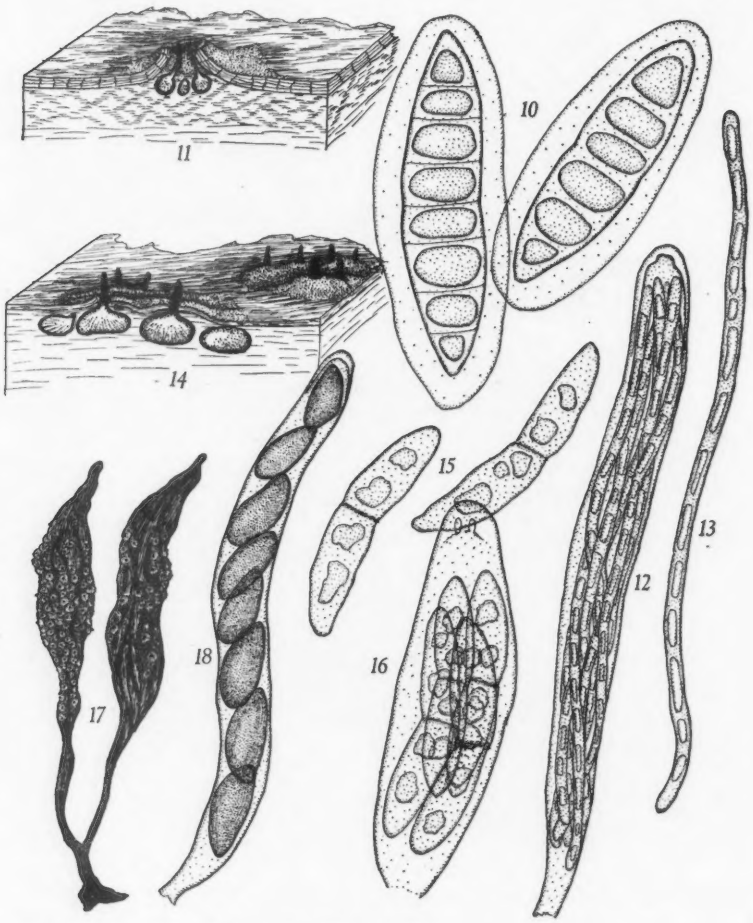


FIG. 10. *Ascospores of Massaria salilliformis Wehm.*

FIGS. 11 TO 13. *Cryptospora aurantiaca Wehm.*
FIG. 11. *Radial section of perithecial stroma.* FIG. 12. *Ascus.* FIG. 13. *Ascospore.*

FIGS. 14 TO 16. *Diaporthe quadruplex Wehm.*
FIG. 14. *Radial section and surface views of perithecial stromata.* FIG. 15. *Ascospores.* FIG. 16. *Ascus.*

FIGS. 17 AND 18. *Xylaria coprophila Wehm.*
FIG. 17. *Habit of perithecial stromata.* FIG. 18. *Ascus with ascospores.*

in the spores of *L. hirsuta* are usually faint or absent in most collections, and this is probably that species. The asci are of the *Bombardia* type.

Pseudotrichia viridicoma (Cke. and Pk.) comb. nov. (*Sphaeria viridicoma* Cke. and Pk. Rept. New York State Museum, 29 : 64. 1878.) On wood infected by some pyrenomycete, Upper Brookside, Aug. 12, 1931 (1174).

The nomenclature of this fungus was treated by the writer (22, p. 57) previously, under the name of *Pseudotrichia aurata* (Rehm.) Wehm. It has since been noted that Kauffman (9, p. 187) discussed the same fungus from northern Michigan and placed it as *Lophiotricha viridicoma* (Cke. and Pk.) Kauff. Kauffman, as did Petrak, emphasized the slit-like character of the ostiole and placed the fungus in the Lophiostomataceae. The ostioles are definitely conic, however, and the fact that the apical opening is sometimes slit-like instead of pore-like is not sufficient reason for placing it in this family. The spores are not of the type found in the true species of *Lasiosphaeria*, as Kauffman recognized. It seems better to retain the fungus in Kirschstein's new genus *Pseudotrichia* which was created for it. The species name *aurata* is preceded by *viridicoma*, however, and a revised synonymy of the species is presented.

Pseudotrichia viridicoma (Cke. and Pk.) comb. nov.

Sphaeria (*Villosae*) *viridicoma* Cke. and Pk., Rept. New York State Museum, 29 : 64. 1878. (also 26 : 87. 1874.)

Lasiosphaeria viridicoma Sacc., Sylloge Fungorum, 2 : 193. 1883.

Thyridaria aurata Rehm., Ann. Mycol. 10 : 392. 1912. (also 12 : 172.)

Lophiotricha viridicoma Kauff., Mich. Acad. Sci. (Papers) 9 : 189. 1929.

Pseudotrichia stromatophila Kirschst., Ann. Mycol. 37 : 125. 1939.

Pseudotrichia aurata Wehm., Mycologia, 33 : 60. 1941.

Kauffman's collection is on decorticated wood which had obviously been infected by some other pyrenomycete, as shown by the blackened zones within, and on the surface of the wood. Reliquae Farlowianae No. 42, of *Lasiosphaeria viridicoma* is also on stromatic disks of one of the Sphaeriales. These collections confirm the opinion that this fungus is of wide occurrence on wood and bark infected by pyrenomycetes and has probably been described under other names.

Melanommeae

Bertia moriformis (Tode) de Not. On decorticated stick, Upper Brookside, July 11, 1931 (495); on *Acer spicatum* Lam., New Glasgow Rd., July 25, 1931 (1152).

Melanomma disjectum (Karst.) Sacc. On decayed log (fir?), Upper Brookside, July 19, 1929 (168).

This collection fits the description of this species almost exactly. The fungus is of the *Leptosphaeria* type, however. The perithecia are immersed within the wood fibres when young and later are erumpent. The spores are fusoid-ellipsoid, pale yellow, straight or slightly curved, 20–22 × 4–4.5 μ and have the second cell from the apex slightly enlarged, as is so often found in *Leptosphaeria*. The paraphyses are septate, cellular strips of interthecial tissue.

Melanomma Pulvis-pyrius (Pers.) Fck. On decorticated wood, Upper Brookside, Aug. 11, 1931 (1275); Victoria Park, July 18, 1933 (1591).

The species of *Melanomma* are difficult to separate. Most collections are lumped under the ubiquitous and variable *M. Pulvis-pyrius* on the one hand, and numerous species have been described with doubtfully valid differences on the other. Winter (24, p. 249), Ellis (5, p. 181), and most authors give the spores of this species as 16–18 × 4–6 μ. Chesters (3, p. 119), after examining a large series of collections, found the spores to vary from 11.5–18 × 4–6 μ, with most spores being 14–16 × 5 μ, and very few below 14 μ in length. No. 1591 has spores 14–16 × 4–4.5 μ, which are typical of this species. In No. 1275 the spores are somewhat smaller (12.5–16 × 3–3.5 μ) and the second cell is usually somewhat swollen. The perithecia are also more hemispheric, and somewhat sunken in the slightly blackened surface of the substratum. It approaches the *Leptosphaeria* type of organization. It was thought that this might be *M. asterostomum* E. and E., on beech roots, but material of this species, kindly sent by Dr. Dearness, shows that species to have more superficial, somewhat elongate perithecia and spores 12.5–14 × 3.5–5 μ, without a swollen cell. No. 1275 might be placed in *M. Aspergrenii* Fck.

Melanomma subsparsum Fck. On dead stick, Victoria Park, July 6, 1931 (409); on bark of *Betula* sp., Upper Brookside, July 10, 1931 (476); Green Oaks, Colchester Co., July 9, 1931 (472); on bark of *Sorbus americana* Marsh., Victoria Co., Aug. 5, 1931 (1244).

Under this name are grouped a number of collections of a sort which are commonly found cited as *M. Pulvis-pyrius*, but in which the spores are definitely smaller than given for this latter species. The spore measurements of these collections are: No. 409, 12.5–13 × 3.5–4.5 μ; No. 476, 12–14.5 × 4–5 μ; No. 1244, 10.5–12.5 × 3.5 μ. These fall within the range given for *M. fuscidulum* by Chesters (3, p. 123), but these collections do not have the prominent ostiole nor the biseriate spores of that species.

Rosellinia conglobata (Fck.) Sacc. var. **microtricha** (Feltg.) Höhn. On *Fagus grandifolia*, Middle River, Victoria Co., Aug. 5, 1931 (1229).

The spores of this collection are 8–10.5 × 4.3–5.5 μ. They are somewhat smaller than those of *R. ligniaria* (Grev.) Fck. and somewhat larger than those of *R. velutina* Fck., although the perithecia have the short stout spines of these species. This material differs from *R. pulveracea* in the spiny perithecia and smaller spores, but Thuemen's Mycotheca Universalis No. 861 of *R. pulveracea* forma *Sambuci-racemosae* shows these short spines, but has the spores of *R. pulveracea* (10.5–12 × 5.5–7.5 μ). Saccardo* gave *R. Sordaria* (Fr.) Rehm. as having spiny perithecia and the spores of *R. velutina* (7–8 × 5 μ), but Winter (23, p. 100) states that *R. Sordaria* has smooth perithecia. Feltgen (6, III, p. 288) described a variety, *microtricha* of *R. Sordaria* with short spines, and spores 7–10 × 3–5.5 μ, on *Fagus*. Höhnel (7, p. 1198) states that this variety is on *Corylus*, has nothing to do with *R. Sordaria*, and is a variety of *R. conglobata*, with similar spores (9–12 × 5–7 μ). An examination of the type material of this variety, kindly sent by Doctor Linder, proves it to be identical with the Nova Scotian material. Its spores are 7.8–9.5 × 4.5–5.5 μ, and the perithecia have similar short spines.

Zignoella aterrima (Fck.) Sacc. On decorticated hardwood, Middle River, Victoria Co., Aug. 5, 1931 (1227).

Perithecia 200–300 μ in diameter, with base somewhat immersed. Spores fusoid, hyaline, one-celled at first, becoming two-celled and four-guttulate, 12.5–16.5 × 2.5–3.5 μ, accompanied by many small ellipsoid, hyaline, one-celled spores, which might be sprout conidia, although no actual budding of the ascospores was seen. The perithecia are accompanied by stiff, upright, dark brown, sparsely septate conidiophores, 5 μ in diameter. These bear, at their tips, clusters of curved, cylindric conidia which are at first one-celled, hyaline, then two-celled, pale brown, and finally four-celled and brown. They are 12.5–18 × 4.5–5.5 μ. This fits the description of *Zignoella aterrima* very well, except that the spores (12 × 4 μ) and conidia (8–10 × 6 μ) are somewhat smaller in that species.

Zignoella Pulviscula (Curr.) Sacc. On wood of *Fagus grandifolia*, Upper Brookside, July 11, 1931 (489); Aug. 11, 1931 (1275).

The spores in these collections are long cylindric-fusoid, usually curved, hyaline, one-celled, many-guttulate, and 25–37 × 3.5 μ. Although not yet mature enough to show septa, their measurements are greater than those given for this species. The wood surface bears stiff, upright, dark brown, septate conidiophores which, in No. 489, bear four to many-celled conidia, which are ellipsoid to cylindric, sometimes curved, and have the central cells coloured brown, whereas the end cells remain hyaline. They measure 21–45 × 7 μ.

Lophiostomeae

Lophidium compressum var. **microscopicum** Karst. On *Betula* sp., Salmon River, July 15, 1931 (1038).

The spores of this collection measure 14–18 (20) × 5.5–6 μ and are rather small for the measurements of this species as given by Winter, but fit those given for the variety. The perithecia are also superficial as given for the variety, and, when young, show a sparse covering of fine short hairs which soon disappear however.

Amphisphaeriaceae

Amphisphaeria Juniperi Tracy and Earle. On weathered wood of fir, Portapique Beach, July 31, 1933 (1603); on rotten wood (conifer?), Victoria Park, June 21, 1933 (1674).

Of a number of Amphisphaerias described on coniferous wood, this collection fits best the above species. The perithecia are conic with a flattened base, 450–500 μ in diameter and have thick parenchymatous walls. The asci are clavate with a thickened apical wall, 105–135 × 25–30 μ. The spores are biseriate, ellipsoid-fusoid, two-celled, brown, constricted at the septum, with the lower cell often smaller and tapered, 32–39 × 11–12.5 μ.

* *Sylloge Fungorum*, 1: 270. 1882.

No. 1674 is accompanied by a conidial stage consisting of upright, superficial, cylindric-conic, black pycnidia with a large ring-like ostiole and a central mass of hyaline conidia budded off from the peripheral walls. The conidia are fusoid-ellipsoid, one-celled, and measure 4.3–5.3 × 1.5–2 μ.

Strickeria obducens (Fr.) Wint. On *Spiraea* sp., Salmon River, Truro, Sept. 3, 1929 (268).

The spores of this collection (23–25 × 7–9 μ) are slightly smaller than those given for *S. obducens*, but this is probably that species.

Strickeria vilis (Fr.) Wint. On *Fagus grandifolia*, Upper Brookside, July 1, 1931 (351).

The perithecia of this specimen are small, 200–250 μ in diameter, and erumpent in small clusters through pustulate ruptures of the bark surface. The spores are three-septate, constricted at the central septum, with a longitudinal septum dividing the two central cells, 12.5–15 × 5.5–6 μ; the lower half of the spore is more pointed than the upper rounded half. The fungus is somewhat intermediate between *Strickeria*, *Pleospora*, and *Cucurbitaria*, but seems to fit this genus and species best.

Trematosphaeria callicarpa Sacc. On decayed wood, Folleigh Lake, June 21, 1926 (*P*380).

The very large, eight-celled brown spores (75–89 × 14–16 μ), with the cells from the central septum toward the ends becoming successively smaller, until the apical cells are small, cap-like, and pale, are characteristic of the species.

Trematosphaeria faginea Morg. On *Fagus grandifolia*, Salmon River, Sept. 7, 1931 (1480); Upper Brookside, June 27, 1933 (1564).

The spores of this species are one-celled, brown, and four-guttulate at first, but become three-septate at maturity. The barely immersed perithecia appear as thickly scattered, blackened, hemispheric pustules on the bark surface.

Ceratostomeae

Ceratostoma parasiticum E. and E. On *Fomes fomentarius* (L.) Gill., Folleigh Lake July 20, 1931 (1106).

Mycosphaerellaceae

Mycosphaerella colorata (Pk.) Earle. On leaves of *Kalmia angustifolia* L., Upper Brookside, July 22, 1929 (105); Portapique Beach, Aug. 7, 1933 (1617).

The spots caused by this fungus are at first red-brown with darker raised margins, as given for *M. colorata*. Later the centres bearing the perithecia are dead and grayish. The spores of these collections were 11.5–14.5 × 2 μ and somewhat curved. The spores of *M. colorata* are given as 14–18 × 2.5 μ. *Sphaerella brachytheca* Cke., on *Vaccinium Vitis-idaea* L., has similar spots and smaller spores (14–15 × 3 μ). There are other species of *Sphaerella* described on Ericaceae which are very similar. Ellis's N.A.F. No. 899, of *Sphaerella colorata* Pk., has identically formed spots but only immature asci could be found in this collection.

Mycosphaerella Coptis (Schw.) House. On leaves of *Coptis groenlandica* (Oeder) Fern., Moore's Lake, Halifax Co., July 6, 1929 (32).

The spores of this collection are immature, 9–12.5 × 2.5 μ, and still one-celled and four-guttulate, but apparently will become two-celled. The material agrees well with Ellis's N.A.F. Nos. 2358 and 2359 of this species.

This collection is accompanied by a species of *Septoria* with spheric pycnidia, 100–150 μ in diameter, sunken in the circular grayish spots which are surrounded by a purplish-black discoloration. The conidia are acicular, 40–71 × 0.5–1 μ. This is probably *Septoria Coptidis* B. and C., although Berkeley gives the spots caused by his species as rufous with a red-brown margin and the spores as 25 μ long.

Mycosphaerella punctiformis (Pers.) Schroet. On leaves of *Fagus grandifolia*, Deep Hollow, Wolfville, June 25, 1926 (*P*372), mostly sterile material.

Mycosphaerella Sarraceniae (Schw.) House. On leaves of *Sarracenia purpurea* L., Castle Rey Lake, Aug. 10, 1933 (1626).

Mycosphaerella Virgaureae Krieg. On stems of *Solidago* sp., Upper Brookside, June 19, 1933 (1522).

Of the species of *Mycosphaerella* on *Solidago*, *Sphaerella nebulosa* (Pers.) on stems is described as having a superficial appearance identical with this collection, but the spores (14–16 × 5 μ) and asci (50 × 15 μ) are larger. *M. Virgaureae* has a similar appearance on leaves with asci 27–30 × 5 μ and spores 10–15 × 2–3 μ. The asci of this collection are 26–35 × 7–9 μ and the spores 10–11 × 2.5–3 μ.

Phaeosphaerella pheidasca (Schroet.) Sacc. On *Juncus* sp., Victoria Park, June 26, 1935 (1706).

Pleosporaceae

Didymella tosta (Berk. and Br.) Sacc. On *Epilobium angustifolium* L., Victoria Park, July 18, 1933 (1589).

Petrak (14, p. 237) has made this species the type of a new genus, *Paradidymella*, which includes those species of *Didymella* with a sphaeriaceous type of perithecium.

Didymosphaeria populifolia E. and E. On leaves of *Populus tremuloides* Michx., Salmon River, July 7, 1933 (1579).

This collection agrees with the type (Dearness No. 2263, 1894) of this species, kindly sent by Dr. Dearness. This may be a synonym of *Phaeosphaerella macularis* (Fr.) Trav., for the asci are fasciculate and without paraphyses.

Didymosphaeria Thalictri Ell. and Dearn. On *Thalictrum* sp., Folleigh Lake, June 21, 1926 (*P*366); Victoria Park, June 26, 1933 (1556).

Leptosphaeria

This genus is a most interesting one. Many of the species show minor differences of spore structure, often correlated with the host. In the descriptive literature, these details are not always given. Large numbers of species have been described, largely on host distinctions, on the one hand, whereas many host varieties have been obscured by inclusion in one of the ubiquitous species on the other hand. As a result it is difficult to be sure of species determinations without a comparative study of the entire genus. In order to facilitate such comparisons, descriptions of the spores are given for the collections here discussed.

Leptosphaeria anisomeres sp. nov. (Figs. 3 to 5).

Appearing on the surface as effuse, splotched, or blackened areas of the leaf sheath, through which the minute papillate ostioles are erumpent, each through a minute swelling caused by the immersed perithecium, but scarcely visible without a strong lens. Perithicia clustered or densely crowded, 150–200 × 100 μ, formed beneath the epidermis and surrounded by a proliferation of dark brown hyphae which cause the blackening of the surface. Walls rather thin and equal in thickness. Asci clavate with a tapering base and a slightly thickened apical wall, 50–60 × 6–7 μ. Spores biseriate above, uniseriate below, fusoid, inequilateral to slightly curved, three- to five-septate, not constricted at the septa, yellow-brown, 12.5–14.5 × 2.5–3.5 μ. The young spore is four-celled with the upper two cells (6–6.5 μ) shorter than the lower (7.5–8 μ). Either one of the larger (lower) cells, or both, may form a second septum.

Type: Herbarium L. E. Wehmeyer, on *Agropyron repens*, Upper Brookside, Colchester Co., N.S., 22, VI, 1933, leg. L. E. Wehmeyer (No. 83).

The proliferation of dark hyphae about the perithecia resembles that found in *Kalmusia*, but no species with small spores and this peculiar type of septation could be found in either genus on Gramineae.

Leptosphaeria anisomeres sp. nov. Superficialiter effusa, maculosa, nigrescens, obvia in foliorum vaginis *Agropyronis repentis*; ostiolis minutis (solum sub lente vix visibilibus), papilliformibus erumpentibus ab areolis minutis elevatis circumdatis; peritheciis immersis, plus minusve dense aggregatis, diametro 150–200 μ, altitudine 100 μ, subepidermalibus et circumdatis a hyphis atrobrunneis superficiem vaginae nigricantibus; membranis tenuisculis equalibus; ascis clavatis, basi angustatis, apice membrana subincrassata praeditis, 50–60 longis, 6–7 μ crassis; sporis in parte ascorum superiore biseriatis, in parte inferiore uniseriatis, fusoideis, inaequilateralibus vel paulum curvatis, 3- vel 5-septatis, ad septa haud constrictis, luteo-brunneis, 12.5–14.5 μ longis, 2.5–3.5 μ crassis, juventate saepissime 4-cellulis, cellulis 2 superioribus quam 2 inferioribus brevioribus, superioribus nunquam dividentibus, aetate interdum 5- vel 6-cellulis a divisione cellularum inferioribus singulis vel ambabus.

Specimen typicum in auctoris herbario conservatum, legit L. E. Wehmeyer (No. 1683) in loco dicto "Upper Brookside, Colchester Co." in Nova Scotia, 22, VI, 1933.

Proliferatione hypharum nigrescentium circum perithecia *Kalmusiae* similis, sed a speciebus omnibus *Kalmusiae* et *Leptosphaeriae* gramina infestantibus differt sporis parvis inaequabiliter septatis.

Leptosphaeria Coniothyrium (Fck.) Sacc. On *Rubus* sp., Victoria Park, July 6, 1931 (407); Upper Brookside, Aug. 11, 1931 (463).

Associated with *Pyrenopeziza Rubi* (Fr.) Rehm. Spores fusoid, four-celled, inequilateral or curved, constricted at the central septum and sometimes less so at the other septa, end cells somewhat longer than the central cells, 12–16 × 3–4 μ. This collection differs from those placed under *L. dumetorum* chiefly in the shorter spores and crowded perithecia, causing a blackening of the stem surface.

Leptosphaeria Doliolum (Pers.) Ces. and de Not. On *Solidago* spp., Upper Brookside, Aug. 1, 1929 (207); Victoria Park, Aug. 3, 1929 (267); Salmon River, Aug. 1, 1931 (1208); Aug. 1933 (1650); on *Aster* spp., Upper Brookside, July 15, 1929 (74); Victoria Park, June 26, 1933 (1559).

This most widespread species has the thick, "stromatic", perithecial wall typical of the Pseudosphaeriaceae. The asci are rather long-cylindric, 75–90 × 6–7 μ, and the spores are characteristically fusoid, slightly curved or lunate, four-celled, rather dark brown, not constricted at the septa, or very slightly so in age, 17–25 × 4.5–5 μ.

Leptosphaeria dolioloides (Auersw.) Karst. On *Tanacetum* sp., June 26, 1933 (1541).

The spores of this collection are long-fusoid, pale greenish-yellow, slightly curved, nine-celled, not constricted at the septa but with the fourth cell swollen, and 40–44 × 4–4.5 μ. These spores are more constant in their septation and greater in diameter than given for this species, which may be a mixture of varieties.

Leptosphaeria Dumetorum Niessl. On *Clematis* sp., Salmon River, Aug. 1, 1931 (1207); on *Sambucus pubens*, June 27, 1935 (1708).

Under this species name are placed two collections with four-celled, fusoid, yellow-brown spores, 16–19.5 × 3.5–4.5 μ which have the second cell slightly enlarged. The perithecia are more globose and thinner-walled than the "stromatic" type of *L. Doliolum* and the spores are more constantly constricted and at the lower end of the range of measurements for that species.

The collection on *Clematis* has thickly scattered globose perithecia with rather prominent conic ostioles. The spores are inequilateral or somewhat curved, constricted at the central septum, and with the second cell only occasionally or very slightly swollen. Most species reported on *Clematis* have much larger spores. *L. pyrenopezizoides* Sacc. and Speg. with similar spores differs in the smaller perithecia ($\frac{1}{6}$ mm.) which collapse pezizoid.

On *Sambucus*, the perithecia are more scattered with less prominent ostioles. The spores are old, more strongly constricted at all septa, almost straight, and with a more prominent second cell. There are occasional small spores which are without constrictions and which measure 12–17 × 3.5–5.5 μ.

Leptosphaeria Ellisiana Berl. On *Oenothera* sp., Portapique Beach, Aug. 3, 1933 (1609).

The spores of this collection are fusoid, somewhat curved, four-celled, strongly constricted at the central septum, constricted at the remaining septa when fully mature, and 28–35 × 5–6 μ. They are characterized by a slight asymmetry in which one-half of the spore is somewhat broader and more rounded at the tip and the other half is slightly narrower and more tapered.

Leptosphaeria Onagrae Rehm. is this same species. Rehm. Asc. No. 2080 of *L. Onagrae*, on *Onagra* (*Oenothera*) *strigosa* (Rydb.) Mack. and Bush, has spores which are slightly smaller (26.5–31 × 4.5–5 μ) and show a more pronounced asymmetry than spores of the type collection (Ellis's N.A.F. No. 697 of *Sphaeria subconica*) of *L. Ellisiana*. Unless this is correlated with the host, however, it lies within the specific range of variation.

Leptosphaeria herpotrichoides de Not. On *Poa* sp., Victoria Park, June 23, 1926 (*P*363); on *Agropyron repens*, Upper Brookside, June 22, 1933 (1670 and 1683).

These three collections on grass culms have similar cylindric-fusoid spores which are six- to seven-septate and have the third cell somewhat swollen. The collections on *Agropyron* have spores somewhat shorter (19.5–26.5 × 3.5–4.5 μ) than those found on *Poa* ((24) 28–35 × 4–5 μ) which are more variable. This fungus on *Agropyron* might be considered as *L. culmifraga* var. *bromicola* Bres.

Leptosphaeria Kalmiae Pk. On *Kalmia angustifolia*, Portapique Beach, Aug. 7, 1933 (1616 and 1621).

The spores of these collections approach the *Melanomma* type. They are fusoid-ellipsoid, yellow-brown, four-celled, straight or slightly curved, constricted at the central septum and later at all three septa, 13–17 × 4–5.5 μ. The spores are two-celled at first and one-half of the spore is often broadly rounded, with the other half more tapered.

Leptosphaeria Millefolii (Fck.) Niessl. On *Achillea millefolium* L., Upper Brookside, June 23, 1933 (1532).

The spores of this collection are long-fusoid, yellow-brown, eight-celled, tapered equally toward either end, constricted at the central septum, and with the cell on either side of the constriction definitely enlarged. They measure 34–44 × 4–5 μ.

Leptosphaeria ogilviensis (B. and Br.) Ces. and de Not. On *Solidago* sp., Upper Brookside, June 18, 1926 (*P*361); on *Chrysanthemum* sp., Victoria Park, Truro, June 26, 1933 (1557).

Various species of *Leptosphaeria* may be found in the exsiccati under this binomial. The one most generally found and fitting most descriptions, as far as they go, has a very characteristic spore. These spores are long-fusoid, slightly curved, light yellow-brown, six-celled, somewhat broader and constricted at the central septum and 30–38 × 3.5–4.5 μ. One end of the spore tapers gradually to a narrow tip; the other has a rounded apex, is somewhat narrower in diameter just below this apex, and widens again to a greater diameter in the region of the central septum. The spores of the collection on *Chrysanthemum* are of this type. Those on *Solidago* are smaller (25–32 × 2.5–3.5 μ) and do not show this asymmetry. These may be merely immature spores, but such spore differences are often constant in this genus.

Leptosphaeria planiuscula (Riess) Ces. and de Not. On *Solidago* spp., Onslow Marsh, Truro, June 19, 1926 (*P*365); June 19, 1933 (1519 and 1520); Portapique Beach, June 31, 1933 (1604); Victoria Park, June 26, 1933 (1654); on *Aster* spp., Upper Brookside, July 27, 1931 (1160 and 1161).

These collections have spores which are fusoid, yellow-brown, straight or slightly curved, symmetrical, four-celled at first, becoming six-celled, constricted at the central septum at first, finally constricted at all the septa, 50–67 × 7–9 μ. The spores often show faint cap-like protoplasmic appendages at the ends.

Leptosphaeria rhopalispora Berl. On *Gnaphalium* sp., Victoria Park, June 26, 1933 (1542); Salmon River, July 7, 1933 (1574).

The spores of these collections are clavate-fusoid, rounded at one end and tapered toward the other, yellow-brown, slightly curved, four-celled at first, then six-celled, 25–33 × 4–5 μ, and constricted at the septa only at full maturity. They fit the description and Berlese's figures of this species on *Inula*, but no material has been seen for comparison.

Leptosphaeria vagabunda Sacc. On *Solidago* sp., June 19, 1933 (1520).

L. Doliolum occurs on these same stems, but the perithecia of this species are more globose and thinner walled and the asci (82–90 × 14–16 μ) are more broadly clavate. The spores are more crowded, biseriate, ellipsoid, straight or slightly curved, four-celled, more strongly constricted at the septa, often with the second cell slightly enlarged, and measure 21–26 × 7–8.5 μ.

Ophiobolus acuminatus (Fr.) Duby. On *Cirsium* sp., Victoria Park, June 26, 1933 (1544); Salmon River, July 7, 1933 (1575).

The spores of this species are many-celled, 110–135 × 2–3 μ and with one swollen cell excentrically placed, or with two adjacent cells swollen at the ends opposite the common septum. The spores tend to constrict at the septa and fall apart into biguttulate cells. Berlese (1, p. 88) created the genus *Leptosphaeriopsis* for those species which had 16-spored asci, with the spores attached in pairs, by their ends, and included this species therein. This is merely a roundabout interpretation, of course, of the fact that the spores tend to fall apart at the middle between the two swollen cells.

Ophiobolus porphyrogonous (Tode) Sacc. On *Corallorhiza maculata* Raf., Wolfville, June 25, 1926 (*P*249*b*); on *Spiraea* sp., Salmon River, Sept. 3, 1929 (269).

Physalospora Laricis sp. nov. (Figs. 6 to 8).

Perithecia spheric, 400–500 μ in diameter, somewhat flattened or concave on top with a central papillate ostiole, thickly scattered, singly erumpent through the periderm, eventually falling out and leaving a circular perforation, white within, walls 50–80 μ thick, composed of large, dark-brown–walled pseudoparenchyma. Asci large, stout-clavate, with a short stipe and a claw-like base, apical wall strongly thickened, (90) 115–150 × 26.5–32 μ, embedded in an interthecial tissue of paraphysis-like strands. Spores biseriate, ellipsoid to fusoid-ellipsoid or inequilateral, one-celled, brown, often with a disk-like refractive body in the centre of the spore, 34–41 × 12–14 μ.

Type: Herbarium L. E. Wehmeyer, on *Larix laricina*, Upper Brookside, Colchester Co., N.S., 8, VII, 1931, leg. L. E. Wehmeyer (No. 434); on *Larix*, Salmon River, N.S., 3, VII, 1931 (No. 374).

This is a typical *Physalospora*, the perithecia being thick-walled and pseudosphaeriaceous in structure with a small pore-like ostiole, but the spores are definitely brown. Theissen would use this genus name for sphaeriaceous forms, whereas Höhnel uses it for the pseudosphaeriaceous ones. The original generic description allows the inclusion of coloured spores. It seems strange that this striking fungus has not been described, but no fitting description could be found in any likely genera. It does not have the clypeus

of *Anthostomella*, and *Paranthostomella*, without a clypeus, contains no similar species. The genus *Maurinia* is supposed to have asci with a plug in the ascus tip which stains blue in potassium iodide, which is not true of this collection. *Phaeobotryon* (*Phaeobotryosphaeria*) has compound stromata.

Physalospora Laricis sp. nov. Perithecia sphaerica, 400–500 μ diametro, apice applanata vel concava et ostiolo centrali papilliformo praedita, densiuscule dispersa, singula per peridermate erumpentia, intus alba, demum delapsu perforationem circularem relinquentia; parietibus 50–80 μ crassis, parenchymatis, atrobrunneis, grandicellulis; ascis magnis, breviter clavatis, breviter stipitatis, basi deflexis, apice valde incrassatis, 115–150 μ longis, 26.5–32 μ crassis, inter filamenta interthecialia paraphysiformia immersis; sporis biseriatis, ellipsoideis vel fusiformibus vel inaequilateralibus, 1-cellulis, bruneis, saepe somate disciformi refractivo centrali praeditis, 34–41 μ longis, 12–14 μ crassis.

Specimen typicum in auctoris herbario conservatum, in ramis ramulisque *Laricis laricinae* prope locum dictum "Upper Brookside, Colchester Co." in Nova Scotia, legit L. E. Wehmeyer (No. 434), 8, VII, 1931; etiam in *Larice*, Wehmeyer No. 374, ad "Salmon River" in Nova Scotia, 3, VII, 1931.

Pleospora herbarum (Pers.) Rab. On *Lathyrus japonicus* Willd., Portapique Beach, Aug. 3, 1933 (1672).

Pleospora nitida (E. and E.) comb. nov. (*Teichospora nitida* E. and E. Proc. Acad. Natural Sci. Phila.). On *Rubus* sp., Wolfville, June 25, 1926 (*P*359).

This collection fits the description of *Teichospora nitida* E. and E. and agrees with the Nuttall collection (Fl. Fayette Co., W. Va. No. 1817) cited by Ellis. As stated in the description, however, the perithecia are formed beneath the periderm and exposed by the exfoliation of this tissue. It belongs properly in the genus *Pleospora*.

Venturia curviseta Pk. On leaves of *Nemopanthus mucronatus* (L.) Trel., Grande Anse, Richmond Co., Aug. 3, 1931 (1236).

The spores of this collection are brown when fully mature, and 8.5–11 × 4–5.5 μ. The asci are cylindric, 53–60 × 5–7 μ. The perithecia are 100–150 μ in diameter and have the typical recurved setae.

Venturia Gaultheriae E. and E. On leaves of *Gaultheria procumbens* L., Upper Brookside, July 14, 1929 (48).

Venturia inaequalis (Cke.) Wint. On leaves of *Malus pumila* Mill., Kentville, Kings Co., March 27, 1927 (116), perithecia immature.

Gnomoniaceae

Apiognomonia guttulata (Starb.) comb. nov. (*Gnomoniella guttulata* Starb. Ascom. Oeland, p. 10). On *Agrimonia* sp., Victoria Park, Truro, Sept. 7, 1929 (291); June 23, 1933 (1538).

The original description gives the spores of this species as cylindric-fusoid, inequilateral to curved, narrowed downward, with a spurious septum below the middle, 6–10 × 1–2.5 μ. This septum is usually present and visible and the lower cell is usually smaller than the upper. The species, should be, therefore, in the genus *Apiognomonia*.

Gnomonia rostellata (Fr.) Wehm. On *Rubus* sp., Upper Brookside, July 21, 1929 (109).

As pointed out by the writer (19, p. 266), this species should be in the genus *Gnomonia* rather than *Diaporthe*. *G. Rubi* Rehm. is probably the same species on leaves.

Cucurbitariaceae

Gibberidea abundans (Dobr.) Shear (*Naumovia abundans* Dobr.). On *Lycopus americanus* Muhl., Wolfville, June 26, 1926 (*P*373); on *Prunella vulgaris* L., Upper Brookside, July 4, 1931 (1400).

Shear (17, p. 358) is correct in all he says concerning the dothideaceous character of this fungus, and its close relationship to *Rosenscheldia* Speg. and *Gibberidea* Fck. The general citation of the spores of these genera as many-celled and brown, without qualifications, is misleading, inasmuch as the spores are commonly immature, one-celled, and hyaline when collected and become definitely septate and pale brown only at full maturity. The spores of these collections were one-celled, hyaline, and 26–30 × 1.5 μ in No. 400 and hyaline to pale brown, one-celled, and 33–39 × 1.5–2 μ in No. *P*373.

Probably all of the Cucurbitariaceae and numerous other forms now placed in the Sphaeriales have what is considered a dothideaceous structure. This species is retained in this family for the present.

Gibberidea alnea (Pk.) comb. nov. (*Cucurbitaria alnea* Pk. Rept. New York State Museum, 28: 75. 1878.). On *Alnus* sp., Green Oaks, Colchester Co., July 12, 1929 (180).

Appearing on the surface as clusters of spheric, brown-black perithecia, confluent or seated on a subdothideaceous stroma erumpent through lateral ruptures of the periderm. The perithecia are 300–400 μ in diameter, and in sections whitish within. Asci long-clavate with a tapering base, spore-bearing portion 85–100 × 8.5–10 μ; stalks 15–25 μ. The spores are irregularly biseriate, fusoid-ellipsoid, hyaline, four-guttulate at first, then two-celled with a swelling above the septum, and finally four-celled, with the second cell enlarged and a large guttule in each cell, eventually pale brown at maturity, 20–25 × 5–7 μ. When fresh the spores often show short, hyaline, apical appendages containing two refractive granules. Paraphyses numerous, hyaline, filiform, persistent.

Peck (12, p. 75) described his *Cucurbitaria alnea* as having uniseriate, uniseptate, hyaline, two- to four-guttulate spores, 20–25 μ long. It seems probable, however, that he had a young stage of this same fungus. The structure of this material is the same as that of the preceding species, except for the somewhat broader, more definitely septate and constricted spores, which are of the same type as those of *Pseudotrichia aurata* and many other fungi scattered throughout the Sphaeriales. It should be placed in the genus *Gibberidea* as outlined by Shear. *Gibberidea alnicola* Rehm. has smaller spores (12–15 × 4.5–5 μ).

Massariaceae

Massaria inquinans (Tode) Ces. and de Not. On *Acer spicatum*, Upper Brookside, July 4, 1931 (395); Sept. 4, 1931 (1685).

Massaria pruni Wehm. (*M. occulta* (Schw.) E. and E.). On *Betula papyrifera* Marsh., Upper Brookside, July 13, 1931 (500); on *Prunus* (?), July 13, 1931 (1007); on *Amelanchier*, Portapique Beach, July 26, 1933 (1597).

This name change was made by the writer (21, p. 131) because of the pre-existing *Massaria occulta* Romell. There is a legitimate doubt as to the hosts of these collections and they may all be on *Amelanchier*. The spores of these collections are hyaline at first but soon turn brown; they measure 58–64 × 14 μ in No. 500; 60–69 × 14–16 μ in No. 1007 and 63–71 × 13–14.5 μ in No. 1597. The type collection, on *Prunus*, has somewhat smaller spores (52–60 × 12–13 μ) which were all hyaline (although Ellis states that the spores turn brown). It may be that this is a variety on *Amelanchier*, as the species of *Massaria* show minor differences correlated with the host, but a comparative study of more material on these hosts must first be undertaken.

Massaria (*Massarina*) **salilliformis** sp. nov. (Fig. 9 and Fig. 10).

This appears on the surface as thickly scattered blackened areas, 2–5 mm. in diameter, each of which consists of a crowded group of minute papillate pustules which are soon perforated by the black punctate mouths of the separately erumpent ostioles. Perithecia 300–400 × 250 μ, flask-shaped, with thick (30–40 μ) black walls, immersed in the unaltered bark and erumpent directly through the surface by a short ostiolar neck. Asci stout-clavate with a thickened apical wall, (100) 170–210 × 23 μ, embedded in a mass of filiform, hyaline, persistent paraphyses. Spores biseriate, cylindric-fusoid, hyaline, ends blunt, 6- to 10-celled or guttulate, not constricted at the septa, (35) 40–47 × 10–12 μ.

Type: Herbarium L. E. Wehmeyer, on *Fagus grandifolia* bark, Salmon River, Colchester Co., N.S., 7, IX, 1931, leg. L. E. Wehmeyer (No. 1684); on living bark of *Fagus*, Upper Brookside, 1, VII, 1931 (No. 350).

This species is placed provisionally in the genus *Massaria*. The entire family of the Massariaceae needs revision badly, as it contains a number of distinct groups related to scattered species in many genera. This collection belongs to a group typified by long cylindric-fusoid, many-celled, hyaline, non-constricted spores. It is difficult to say where it might have been described previously, but no similar species could be found in *Massaria*, *Massarina*, *Metasphaeria*, or *Calospora*.

Massaria (*Massarina*) **salilliformis** sp. nov. In areis superficialibus *Fagi* ramulorum nigrescentibus densis 2–5 mm. diametro, pustulae minute papillatae dense aggregatae mox perforatae a ostiolis nigris separate erumpentibus; perithecis 300–400 μ altis, 250 μ crassis, ampulliformibus pariete 30–40 μ crasso, nigris, in cortice sano immersis et per orem brevem singulis erumpentibus; ectostromate nullo; ascis breviter clavatis apice incrassatis 170–210 μ longis, ca. 23 μ latis, inter paraphyses persistentes hyalinos filiformes immersis; sporis biseriatis, cylindrice fusiformibus, hyalinis, utrinque obtusis, 6–10-cellulis vel guttulatis, non-constrictis, (35) 40–47 μ longis, 10–12 μ crassis.

Specimen typicum conservatum in auctoris herbario, legit L. E. Wehmeyer (No. 1684), 7, IX, 1931. Habitat in cortice *Fagi grandifoliae* ad "Salmon River, Colchester Co." in Nova Scotia; "Upper Brookside", Nova Scotia (Wehmeyer No. 350) in cortice *Fagi* viventis.

Allantosphaeriaceae

Diatrype Stigma (Hoffm.) Fr. On *Betula papyrifera*, Upper Brookside, July 8, 1931 (431), common.

Diatrypella

The species of *Diatrypella* are difficult to separate. The character of the stroma has been used to separate species, but this is affected by the type of bark and the manner of growth. Ascus size, although probably a diagnostic character, is affected by the variable length of the stalk, the variable distribution of the spores in different asci of different age, when the spore-bearing portion is measured, and the often overlooked empty tip of the ascus. The diameter of the ascus is probably the most dependable measurement. The spores of different species (?) have such overlapping ranges that these must also be used with caution. Perhaps the clearest way to present the facts of these collections is to tabulate the characters of the relevant species and those of the collections concerned. The ascus measurements are taken from the tip to the narrowed basal stalk (spore-bearing portion).

Collection	Stroma	Measurements, μ		Host
		Asci	Spores	
D. decorata	Ovoid	40–48 × 5	5–6 × 1	*Betula*
D. discoidea	Ovoid to discoid	Not given	5 × 0.75–1	*Betula*
D. favacea	Ovoid	70–100 × 9–12	6–8 × 1.5	*Betula*
D. Tocciaeana	Angular discoid	100–120 × 12	5–7 × 1	*Betula and Alnus*
D. nigro-annulata	Angular discoid	100–180 × 10–12	6–8 × 1.5	*Fagus*
D. Demetrionis	Discoid	35 × 6	4–5 × 1–1.5	*Salix*
No. 203	Discoid	37–45 × 5–5.5	3.5–4.3 × 0.6–0.8	*Prunus*
No. 104	Discoid	30–40 × 4–6	3.5–5 × 0.8	*Betula*
No. 477	Discoid	35–43 × 5–5.5	4.3–5 × 0.5–0.8	*Betula*
No. 286	Angular discoid	80–110 × 8.5–10	4.5–6 × 1–1.2	*Alnus*
No. 1463	Angular discoid	88–125 × 7–9	5–6 × 1	*Fagus*
No. 397	Ovoid	Not seen	5–6 × 1–1.5	*Betula*
No. 1675	Ovoid	60–70 × 7–9	4–6 × 1–1.5	*Betula*
No. 1154	Ovoid	50–70 × 11–12	4–6 × 1	*Betula*

From these data, these collections might be distributed as given below. Whether these are all good species is another question.

Diatrypella betulina Pk. On *Betula papyrifera* and *Betula* spp., Upper Brookside, July 13, 1926 (98 and 106); Princeport, July 9, 1931 (469); Victoria Park, Aug. 15, 1933 (1632).

This species is distinguished by the greenish colour of the interior of the stroma.

Diatrypella discoidea Cke. and Pk. On *Betula* sp., Westcooke's Grove, Guysboro Co., Sept. 7, 1925 (104); Upper Brookside, July 10, 1931 (477); on *Prunus* sp., Upper Brookside, July 30, 1929 (203).

This group of collections with short and narrow asci, small, narrow spores (less than 1 μ in diameter) and small discoid stromata, are arbitrarily placed in *D. discoidea*. In his description, Peck (12, p. 71) merely says "asci small." *D. Demetrionis* E. and E. fits these collections very well except for the greater diameter of the spores. *D. decorata* Nit. of Europe is also similar, but with larger spores and laterally elongate stromata.

Diatrypella favacea (Fr.) Nit. On *Betula* spp., Wolfville, June 25, 1926 (397); New Glasgow Rd., July 25, 1931 (1154); Victoria Park, Aug. 15, 1933 (1675).

These collections have the characteristic, laterally elongate stromata of this species. The spores of these collections, and of most other American collections, are somewhat smaller than the measurements given for this species in Europe, and copied in American descriptions.

Diatrypella nigro-annulata (Grev.) Nit. On *Fagus grandifolia*, Upper Brookside, Sept. 3, 1931 (1463).

This and the following species have small angular pustules with closely adherent periderm. The asci are longer than in *D. favacea* and the spores are about the same size. This collection, on *Fagus*, has punctate ostioles rather than the sulcate type usually found in this genus.

Diatrypella Tocciaeana de Not. On *Alnus* sp., Victoria Park, Sept. 7, 1929 (286).

This collection differs from the previous one chiefly in the host and the sulcate ostioles.

Eutypa milliaria (Fr.) Sacc. On wood surface of *Acer* sp., Salmon River, Sept. 2, 1931 (1458); and of *Cornus alternifolia* L., Upper Brookside, July 30, 1929 (152).

The spores of these collections (5–7 × 1.5 μ) are somewhat smaller than those given for this species. Both show the blackened surface crust. Conidiophores, but no conidia, were present on *Acer*. On *Cornus*, lunate-fusoid, filiform, hyaline, one-celled conidia 16–23 × 0.8–1 μ were found in irregular cavities beneath the blackened surface.

Eutypa spinosa (Pers.) Tul. On *Fagus grandifolia*, Upper Brookside, Aug. 12, 1931 (1284) leg. A. H. Smith.

Eutypella alnifraga (Wahl.) Sacc. On *Alnus* sp., Salmon River, Aug. 1, 1931 (1211); Victoria Park, Aug. 13, 1935 (1781).

Diaporthaceae

Apioporthe anomala (Pk.) Höhn. On *Corylus cornuta* Marsh., Upper Brookside, July 14, 1929 (65).

Apparently parasitic and killing the canes which are then covered with the strongly pustulate stromata of this fungus.

Apioporthe Corni Wehm. On *Cornus alternifolia*, Upper Brookside, July 30, 1939 (148); Victoria Park, June 26, 1935 (1711).

Causing a characteristic orange discoloration of the dead limbs of this host. Both collections are accompanied by the conidial stage, *Zythia aurantiaca* (Pk.) Sacc.

Apioporthe phomaspora (Cke. and Ell.) Wehm. On *Myrica pennsylvanica* Lois., Evangeline Beach, Wolfville, June 26, 1926 (*P*369).

Apioporthe vepris (DeLacr.) Wehm. On *Rubus* sp., Victoria Park, June 23, 1926 (*P*148*b*).

Cryptodiaporthe galericulata (Tul.) Wehm. On *Fagus grandifolia*, Upper Brookside, July 21, 1929 (104).

Cryptodiaporthe salicina (Curr.) Wehm. On *Salix* sp., Onslow Marsh, Truro, June 19, 1933 (1521).

This is associated with the conidial stage, *Discella carbonacea* (Fr.) Berk. and Br.

Cryptospora alnicola Höhn. On *Alnus* spp., Salmon River Marsh, Aug. 3, 1929 (279); Upper Brookside, July 13, 1931 (1005); Portapique Beach, Aug. 6, 1935 (1767).

The species of *Cryptospora* are badly in need of revision. Aside from *C. femoralis* Pk., which is a clear-cut species, the collections here considered on *Alnus* and *Corylus* all have spores and asci which are practically identical in their range of size and structure. These spores are long-cylindric, flexuous, usually non-septate and many-guttulate, but finally showing faint septa. They range in length from 50–88 μ but are comparatively narrow (2–3.5 μ). The collections placed under *C. alnicola* have small but sometimes rather strongly pustulate stromata with a closely adherent periderm. There is nearly always a definite, although sometimes minute, grayish ectostroma and often a grayish entostroma about the perithecia which lie in the upper bark and are often bounded below by a blackened zone of bark tissue. The asci are 50–100 × 7–12 μ and the spores 50–88 × 2–3.5 μ.

The European *C. suffusa* (Fr.) Tul. differs from these American collections on *Alnus* in the broader spores (45–80 × 3.5–5 μ) and the tendency to form polysporous asci of the "*Ditopella*" type, with ellipsoid spores. The above American collections agree with the type of Höhnel's (8, p. 107) *C. alnicola* which was kindly loaned to the writer by Doctor Linder. Höhnel gives the spores as 5- to 10-septate but no septa were seen in the type. Occasional, very faint septa are seen in these collections. The spores of the type are 60–80 × 2–3 μ.

Cryptospora aurantiaca sp. nov. (Figs. 11 to 13).

Stromata forming angular rather prominent pustules, 0.8–1.2 mm. in diameter, with a central radiate rupture of the closely adherent periderm which exposes a dark brown disk through which three to eight minute, short, spine-like ostioles are erumpent. Perithecia 200–300 μ in diameter, crowded in the bark just beneath the periderm and erumpent through a usually well developed, orange-brown ectostroma. Asci stout-cylindric, 88–95 × 9–12.5 μ. Spores parallel or interwoven in the ascus, long-cylindric, non-septate, many-guttulate, curved, 65–88 × 2–3.5 μ.

Type: Herbarium L. E. Wehmeyer, on *Alnus* sp., Portapique, N.S., 9, VII, 1933, leg. L. E. Wehmeyer (No. 1624).

This species is distinguished by the orange-brown ectostromatic tissue which turns bright wine-red in potassium hydroxide. This colour may be paler in younger stromata. *C. suffusa* (Fr.) Tul. and *C. corylina* (Tul.) Fck. are both given as having yellow discolorations of the stroma but the spores of these species are of greater diameter (3.5–5.5μ).

Cryptospora aurantiaca sp. nov. Stromata angulosa pustuliformia prominentia, 0.8–1.2 mm. diametro, ad centrum radiate erumpentia per peridermatem et ectostroma aurantiaca copiosa; ascis crassis, cylindricis, 88–95 μ longis, 9–12.5 μ crassis; sporis in asco parallelis vel intertextis, longe cylindricis, non-septatis, pluriguttulatis, curvatis, 65–88 μ longis, 2–3.5 μ crassis.

Specimen typicum in auctoris herbario conservatum, legit L. E. Wehmeyer (No. 1624) in *Alni* cortice prope Portapique in Nova Scotia, 9, VII, 1933.

A speciebus aliis differt ectostromate aurantiaco-brunneo kali caustici actione laete vinaceo vel in stromatis juvenilibus pallidiuscule rubro. A *C. suffusa* et *C. corylina* differt discoloratione stromatis aurantiaca vel brunnea haud lutea, et sporis minoribus (sporis in speciebus ambabus aliis 3.5–5.5 μ diametro).

Cryptospora Betulae Tul. On *Betula papyrifera*, Upper Brookside, July 14, 1929 (61).

Cryptospora femoralis Pk. On *Alnus* spp., Rifle Range, Truro, June 19, 1926 (*P*367); Salmon River, July 1, 1929 (13); New Glasgow Rd., June 30, 1931 (335); Portapique Rd., July 9, 1933 (1623); Victoria Park, June 27, 1925 (13*a*).

This very common species has characteristic spores with swollen ends.

Cryptospora suffusa var. **nuda** Pk. On *Corylus cornuta*, Black Rock, Shubenacadie River, June 28, 1929 (17); Victoria Park, June 29, 1935 (1715).

These collections on *Corylus* differ from *C. alnicola* only in the host and in the general lack of any grayish ecto- or entostroma or any blackened zone in the bark, resulting in minute disks of clustered spine-like ostioles. Peck (13, p. 58) gives his variety *nuda* as differing from *C. suffusa* as follows, "Stroma not suffused with a yellow dust . . . . . the black circumscribing line is also apparently absent in some cases". He gives the variety as on *Alnus* and *Corylus* and apparently includes *C. alnicola*, as here interpreted, in this conception. His varietal name is used here for the form on *Corylus*.

Diaporthe acerina (Pk.) Sacc. On *Acer spicatum*, Economy Lake, Colchester Co., June 16, 1926 (*P*52*c*); Wolfville, June 25, 1926 (*P*52*d*); Upper Brookside, July 16, 1931 (1069).

Very abundant on this host.

Diaporthe Arctii (Lasch) Nit. On *Aster* spp., Upper Brookside, July 27, 1931 (1161); Portapique Beach, Aug. 13, 1933 (1611); on *Solidago* sp., Victoria Park, Truro, June 23, 1933 (1536); on *Gnaphalium* sp., Salmon River, July 7, 1933 (1574).

The collections on *Aster* and *Solidago* stems show a quite distinct host form which approaches closely to *D. pardalota* (Mont.) Fck. in the elongate stromatic patches of surface blackening, which are limited in size (1–10 × 0.5–1 mm.) and often sharply margined, but again confluent and indefinite.

On *Gnaphalium* the surface blackening is entirely absent or limited to minute spots or streaks about the short spine-like ostioles. An accompanying *Phomopsis* stage on this host consists of elongate, raised, blackened stromatic patches, 0.5–1.5 mm. long, containing irregular cavities and alpha conidia which were fusoid, one-celled, hyaline, and 8.5–10 × 1.5–2 μ.

———— ———— var. **achilleae** (Auersw.) Wehm. On *Achillea Millefolium* L., Upper Brookside, June 22, 1933 (1531).

Diaporthe decedens (Fr.) Fck. On *Corylus cornuta*, Black River Gorge, Wolfville, June 26, 1926 (*P*308*a*); Earltown Rd., Aug. 22, 1931 (308*b*); Upper Brookside, Aug. 28, 1931 (1677).

Occasional blackened zones were seen at the margins of the entostromata in both No. 308*b* and No. 1677.

Diaporthe eres Nit. (*D. valida* Nit.). On *Myrica pennsylvanica* Baddeck, Victoria Co., Aug. 4, 1931 (1240).

This seems to be the first report of *D. eres* on *Myrica* (*D. valida* Nit.) from North America.

Diaporthe impulsa (Cke. and Pk.) Sacc. On *Sorbus americana*, Green Oaks, Colchester Co., July 12, 1929 (181).

Diaporthe linearis (Nees) Nit. On *Solidago* spp., Upper Brookside, July 30, 1929 (153); Aug. 1, 1929 (209); Portapique Beach, Aug. 3, 1933 (1608); Salmon River, Aug., 1933 (1650).

Diaporthe oxyspora (Pk.) Sacc. On *Ilex verticillata* (L.) Gray, Evangeline Beach, Wolfville, June 26, 1926 (*P*364).

Diaporthe quadruplex sp. nov. (Figs. 14 to 16).

Appearing on the surface as minute, black, short, stout, conic to cylindric ostioles which may be erumpent singly but usually occur clustered in longitudinal series. Perithecia flattened-spheric, 300–400 × 200 μ, scattered singly or usually crowded in longitudinal series just beneath the surface and causing a slightly elongate, pustulate swelling. The surface of the bark, beneath the periderm, is blackened locally about the ostioles. No ventral zones in bark or wood. Asci stout clavate with a refractive ring in the apex, four-spored, 47–53 × 10.5–12.5 μ. Spores overlapping biseriate, long fusoid-ellipsoid, somewhat curved, two-celled, hyaline, constricted at the septum, four-guttulate, 22.5–27 × 2.5–5 μ.

Type: Herbarium L. E. Wehmeyer, on *Solidago*, Upper Brookside, Colchester Co., N.S., 1, VIII, 1929, leg. L. E. Wehmeyer (No. 207).

This species has the structure of *D. linearis* but has four-spored asci and spores twice as long as in that species. It may represent a four-spored condition of *D. linearis*. The spores are similar to, but larger than those of *D. semiinsculpta*.

Diaporthe quadruplex sp. nov. In caule *Solidaginis* vel *Astri* visibilis ut ostiola minuta, nigra, brevia, crassa, conica vel cylindrica, solitaria vel uniseriatim gregaria; peritheciis oblate sphaericis, 300–400 μ latis, 200 μ altis, solitariis vel longitudinaliter, infra superficiem seriatis et tumiditatem paulum elongatam efficientibus; cortice subepidermali circum ostiola nigrescente; zonis infraperithecialibus nullis vel in cortice vel in ligno; ascis crassis, clavatis, apice annulo refringenti praeditis, 4-sporis, 47–53 μ longis, 10–12.5 μ crassis; sporis biseriatis inter se obtengentibus; elongate fusiformibus vel ellipsoideis, paulum curvatis, 2-cellulis, hyalinis, ad septum constrictis, 4-guttulatis, 22.5–27 μ longis, 3.5–5 μ crassis.

Specimen typicum in auctoris herbario legit L. E. Wehmeyer (No. 207) prope "Upper Brookside, Colchester Co." in Nova Scotia, 1, VIII, 1929.

Diaporthe racemula (Cke. and Pk.) Sacc. On *Epilobium angustifolium*, Deep Hollow Rd., Wolfville, June 25, 1926 (*P*374); on *Epilobium* spp., Upper Brookside, July 13, 1931 (1012); Victoria Park, Truro, July 18, 1933 (1588).

Diaporthe tessella (Pers.) Rehm. On *Salix* sp., Upper Brookside, June 27, 1931 (306).

Diaporthe tuberculosa (Ell.) Sacc. On *Amelanchier* spp., Portapique Beach, July 26, 1933 (1593); Victoria Park, Truro, June 26, 1935 (1705).

Diaporthe Viburni Dearn. and Bisby var. **spiraeicola** Wehm. On *Spiraea* sp., Salmon River Marsh, Truro, Sept. 3, 1929 (281); Upper Brookside, July 13, 1931 (496).

Melanconis Alni Tul. var. **marginalis** (Pk.) Wehm. On *Alnus* spp., Oldham, Halifax Co., Sept. 5, 1929 (277); Portapique Beach, Aug. 3, 1933 (1607); Mt. Uniacke, June 24, 1935 (1660).

The black conidial masses of the conidial stage of this species (21, p. 27) are common on species of *Alnus* and often accompany the perithecial stage.

Melanconis apocrypta Ell. On *Populus* spp., Salmon River, July 7, 1933 (1578); Upper Brookside, July 9, 1935 (1735).

Melanconis Everhartii Ell. On *Acer spicatum*, Economy River, Colchester Co., Aug. 31, 1927 (35); Earltown Rd., Aug. 22, 1931 (1395).

Melanconis nigrospora (Pk.) Wehm. On *Betula* spp., Victoria Park, Aug. 8, 1929 (219); June 21, 1933 (1527); Aug. 15, 1933 (1630); Upper Brookside, July 3, 1931 (1191); July 9, 1935 (1376).

Melanconis stilbostoma (Fr.) Tul. On *Betula* spp., Upper Brookside, July 8, 1931 (435); July 13, 1931 (1011); Aug. 8, 1931 (429); Victoria Park, Aug. 15, 1933 (1652); Aug. 26, 1935 (1661); Portapique Rd., July 9, 1933 (1622).

This is the commonest pyrenomycete found on down limbs and piled brush of birch. It is commonly accompanied by the blackened masses of the conidial stage, *Melanconium betulinum* Schm. and Kze.

Melanconis thelebola (Fr.) Sacc. On *Alnus* spp., Truro, Sept. 18, 1926 (110); Victoria Park, Sept. 7, 1929 (286); Upper Brookside, July 13, 1931 (1671); June 27, 1935 (286*a*).

Quite common on *Alnus*.

Pseudovalsa longipes (Tul.) Sacc. On *Quercus borealis* Michx. var. maxima (Marsh) Ashe (*Q. rubra*), Oakfield, Halifax Co., Sept. 8, 1933, leg. A. R. Prince.

Pseudovalsa stylospora E. and E. On *Acer spicatum*, Deep Hollow Rd. and Duncan Brook, Wolfville, June 25, 1926 (*P*371 and *P*371*a*); Upper Brookside, July 1, 1933 (1571); Victoria Park, June 29, 1935 (*P*371*b*); on *Acer saccharum*, Upper Brookside, July 15, 1929 (46).

Valsa amphibola Sacc. On *Sorbus americana*, Victoria Park, Sept. 7, 1929 (288); on *Malus pumila*, Victoria Park, Sept. 7, 1929 (292).

The collection on *Sorbus* is associated with a *Cytospora* with numerous radial locules and conidia which are allantoid, hyaline, 3.5–4.5 × 1 μ.

Valsa cincta Fr. On *Amelanchier* sp., Portapique Beach, Aug. 3, 1933 (1606); on *Rosa* sp., Portapique Beach, July 26, 1933 (1598).

The collection on *Rosa* might be considered a variety of this species. It differs from the collection on *Amelanchier* chiefly in the gray rather than brown coloration of the entostroma. *V. leucostoma* var. *Rosarum* Sacc. has smaller spores and smaller disks. These spores on *Rosa* are 12.5–18 × 2.5–3.5 μ.

Valsa etherialis E. and E. On *Acer saccharum*, Victoria Park, Aug. 13, 1935 (1780).

Valsa Kunzei Fr. On *Abies balsamea*, Moore's Lake, Halifax Co., July 6, 1929 (21); Victoria Park, Aug. 8, 1929 (223); July 8, 1935 (21*a*); New Glasgow Rd., July 25, 1931 (1676).

Quite common and found on many coniferous genera throughout the United States and Canada. *V. superficialis* Nit. is probably the same species on pine.

Valsa leucostoma Fr. On *Prunus* sp., Upper Brookside, July 13, 1931 (1008).

Valsa nivea (Hoffm.) Fr. sensu Ellis. On *Populus Tacamahaca* Mill., Victoria Park, Sept. 7, 1929 (290).

This collection has the sharply outlined stromata and minute white disks of this species and the small spores (7–9 × 1 μ) which Ellis (5, p. 484) says are characteristic of the American form. Spore measurements by European authors are 12–14 × 3 μ. Collections of such a large-spored form (spores 10–18 × 2–3 μ) on *Salix* have been sent from Lake Temagami to the writer by Dr. H. S. Jackson, and apparently exists also on this continent. This small-spored form fits Ellis's description of *V. pallida* very well except for the "subferruginous" disk of that species.

Valsa salicina (Pers.) Fr. On *Populus* sp., Upper Brookside, June 28, 1931 (315).

Valsa sordida Nit. On *Salix spp.*, Evangeline Beach, Wolfville, June 26, 1926 (Herb. No. 3001); Salmon River Marsh, Truro, Sept. 3, 1929 (273); on *Populus* sp., Oct. 2, 1926 (100).

Valsa stenospora Tul. On *Alnus* sp., Oldham, Halifax Co., Sept. 5, 1929 (277).

This collection is characterized by the minute white to grayish disks, closely adherent periderm, pulvinate swellings above the perithecia and spores 9–12 × 1.7–2.5 μ.

Valsa truncata Cke. and Pk. On *Alnus crispa* (Ait.) Pursh. var. mollis (Fern.) Fern., Black River Gorge, Wolfville, June 25, 1926 (*P*379); on *Alnus* sp., Salmon River, July 1, 1929 (11); Mount Uniacke, June 24, 1935 (1700).

These collections have truncate-conic stromata with laterally elongate brown to black disks and spores 8.5–11 × 1–1.5 μ.

Anthostoma melanotes (B. and Br.) Sacc. On decorticated wood of *Salix* or *Alnus*, Salmon River, Aug. 1, 1931 (1210).

Fenestella minor Tul. On *Alnus* sp., Salmon River, July 14, 1931 (1017).

Valsaria moroides (Cke. and Pk.) Sacc. On *Alnus* sp., Victoria Park, July 23, 1931 (1124).

Calosphaeriaceae

Calosphaeria minima Tul. On *Cornus alternifolia*, Upper Brookside, July 15, 1931 (1055).

Various similar fungi are undoubtedly placed under this binomial. This collection shows numerous, small, angular, brown pustules with a disk of a few irregular, somewhat elongate ostioles, with no ectostroma. Asci numerous, clavate, short-stalked, persistent in the hymenium, eight-spored, 22–26 × 3.5–4 μ. Spores allantoid, or inequilateral, one-celled, 4–5.5 × 1–1.5 μ. Paraphyses rather broad, tapered above, longer than the asci.

Xylariaceae

Daldinia concentrica Ces. and de Not. On *Alnus* spp., Economy, Colchester Co., July 9, 1926 (94); Green Oak, Colchester Co., June 12, 1929 (221); Salmon River, July 14, 1931 (1019).

Hypoxylon coccineum Bull. On *Fagus grandifolia*, Economy Lake, June 16, 1926 (318*a*); Mt. Thom, Aug. 10, 1931 (318*b*).

Hypoxylon cohaerans Fr. On *Fagus grandifolia*, Folleigh Lake, Sept. 3, 1928, leg. A. R. Prince (6074); Upper Brookside, June 29, 1931 (326).

Common on dead or injured beech, forming irregular black stromatic crusts.

Hypoxylon fuscum Fr. On *Alnus* sp., Rifle Range, Truro, June 25, 1926 (*P*385*a*); Deep Hollow Rd., Wolfville, June 25, 1926 (*P*384); on *Corylus cornuta*, Upper Brookside, July 29, 1931 (446).

Hypoxylon Morsei B. and C. On *Alnus* sp., and *Salix* sp., Victoria Park, July 23, 1931 (1123 and 1128).

Hypoxylon multiforme Fr. On *Betula papyrifera*, Mill Lake, Upper Musquodoboit, Halifax Co., June 14, 1925, leg. A. R. Prince (102); Killag Mines, July 30, 1931 (1189).

Common on birch, usually as reddish fusoid stromata erumpent through laterally elongate ruptures of the periderm.

Hypoxylon rubiginosum (Pers.) Fr. On *Acer spicatum*, New Glasgow Rd., July 25, 1931 (1149 and 1153); Folleigh Lake, Aug. 24, 1931; Upper Brookside, July 18, 1929 (137); on decorticated wood, Middle River, Victoria Co., Aug. 5, 1931 (1226); Mt. Thom, Aug. 10, 1931 (1251).

Quite common and variable in form.

Hypoxylon ustulatum (Bull.) Fr. (*Ustulina vulgaris* Tul.). On *Betula* sp., Mill Lake, Upper Musquodoboit, Halifax, Co., June 14, 1925, leg. A. R. Prince (1134); on mossy log, Middle River, Victoria Co., Aug. 5, 1931 (1228).

Xylaria castorea Berk. On decayed wood of various species, Princeport, Sept., 1927 (6107); Folleigh Lake, Aug. 23, 1927 (6042); Upper Brookside, Sept. 28, 1926 (6050); Oct. 20, 1926 (103); July 4, 1931 (401).

The first three collections were made by A. R. Prince.

Xylaria coprophila sp. nov. (Figs. 17, 18).

Stromata brown-black, 0.5–1.5 cm. tall. Stalk terete to flattened, 0.5–1 mm. in diameter, roughened with longitudinal wrinkle-like ridges; not tomentose. Fertile head flattened, with a pointed apex which remains sterile, 1–1.5 mm. in diameter. Perithecia 250–300 μ in diameter, scattered or densely crowded, base partially sunken in the surface of the stroma or almost superficial. Ostiole rather prominent, conic-papillate. Perithecial wall thin membranous. Asci cylindric, short stipitate, spore-bearing portion 60–70 × 5–5.5 μ; stipe 15–20 μ long. Spores uniseriate, inequilateral, one-celled, dark brown, 9–10.5 × 3.5–4.5 μ.

Type: Herbarium L. E. Wehmeyer, on porcupine dung, New Glasgow Rd., Pictou Co., N.S., VIII, 1931, leg. A. H. Smith (No. 1490).

Material of this collection was sent to Dr. J. H. Miller, who states (in litt.) that it is different from any species known to him on this substrate. The description of *X. graminicola* Ger. fits this material in many respects. Doctor Linder kindly compared this collection with the type of that species in the Farlow Herbarium and reports that *X. graminicola* is distinct in the presence of a dark brown tomentum on the stem, more strongly inequilateral spores, and several other minor differences.

Xylaria coprophila sp. nov. Stromata in stercoribus *Hystricis*, 0.5–1.5 cm. alta, stipite tereti vel compresso longitudinaliter costato, glabro; capite fertili depresso, 1.0–1.5 mm. diametro, apice cacumine sterili praedito; peritheciis 250–300 μ diametro, dispersis vel

dense aggregatis basi in stromate demidio immersis vel fere superficialibus; ostiolis prominulis conice papillatis; membrana peritheciali tenui; ascis cylindricis, breviter stipitatis, parte sporifera 60–70 μ longis, 5–5.5 μ latis; stipite 15–20 μ longo; sporis uniseriatis, inaequilateralibus, 1-cellulis, atrobrunneis, 9–10.5 μ longis, 3.5–4.5 μ crassis.

Specimen typicum in auctoris herbario, legit A. H. Smith (No. 1490) in fimis hystricinis, prope "New Glasgow Rd.," in Nova Scotia, VIII, 1931.

Xylaria Hypoxylon (L.) Grev. On decayed wood, mossy logs, etc., Upper Brookside, Aug. 21, 1931 (1418); Wentworth Valley, Cumberland Co., Aug. 29, 1931, leg. A. H. Smith (1454); Salmon River, Aug. 21, 1931 (1679).

Xylaria polymorpha (Pers.) Grev. On *Picea* sp., Salmon River, near Truro, Oct. 2, 1925, leg. A. R. Prince (6270).

Dothideales

Dibotryon morbosum (Schw.) Theiss. and Syd. On *Prunus virginiana* L., Truro, July 4, 1925, leg. A. R. Prince (1103).

Dothidella Kalmiae (Pk.) Sacc. On *Kalmia angustifolia*, Oldham, Halifax Co., Sept. 5, 1929 (265); Portapique Beach, Aug. 8, 1933 (1621).

This fungus causes a witches' broom effect with stout elongate upright branches bearing dwarfed leaves. These branches are heavily blackened as the stroma develops over the entire surface. Later the minute points of the ostioles and the papillate swellings of the protruding perithecial locules appear. At the time of these collections, the current year's stromata were immature and the previous year's were old and mostly empty. The only spores seen in them were fusoid, hyaline, two-celled, 12–17 × 2–3 μ, and it is not certain that these were not of some parasitic pyrenomycete, although they were seen in apparently normal stromatic locules. Peck gives the spores as unequally two-celled, 10–12 × 5–6 μ, but the gross appearance is the same.

Dothidella Osmundae (Pk. and Clint.) Sacc. On petioles of *Pteritis nodulosa* (Michx.) Niewl., Upper Brookside, July 15, 1929 (15); Aug. 12, 1931 (1664); on *Pteridium aquilinum*, Upper Brookside, July 27, 1929 (141); on *Osmunda cinnamomea* L., Upper Brookside, July 8, 1931 (445).

Endodothella Junci (Fr.) Theiss. and Syd. On *Juncus* sp., Upper Brookside, July 4, 1931 (399); Deep Hollow Rd., Wolfville, June 25, 1926 (107).

Phyllachora Wittrockii (Erikss.) Sacc. On *Linnaea borealis* L. var. *americana* (Forbes) Rehd., Victoria Park, June 4, 1931 (403); July 22, 1933 (1555).

This is a very striking parasite. The upright tips of *Linnaea* are attacked and the entire stem for a distance of one to two inches is surrounded by a blackened pseudoparenchymatous stroma 300–500 μ in thickness. This stroma is at first wrinkled and then punctate with the minute ostiolar openings. All of the collections made contained only immature perithecial locules without spores. The ascospores apparently mature late in the fall, or, more likely, early spring. The young stromata are covered with a grayish bloom which is the conidial stage consisting of numerous stout, spine-like, pointed conidiophores which bear at their apex a single ellipsoid, one-celled, hyaline to pale brown conidium, 9–9.5 × 3 μ.

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PROTOPLASMIC CONTINUITY IN THE POWDERY MILDEW *ERYSIPHE GRAMINIS* DC.[1]

By Harold J. Brodie[2]

Abstract

The protoplasm in the mycelium and conidiophores of the barley mildew (*Erysiphe graminis* DC.) is continuous from cell to cell. Each transverse septum is provided with a minute central pore through which passes a delicate strand of cytoplasm 1–1.5 μ in width. The cytoplasmic strands and the septal pores have been readily demonstrated by means of a slight modification of Wahrlich's technique, and their presence recorded by means of photomicrographs and drawings. The cytoplasmic connections have also been seen in living unstained mycelium.

Streaming of cytoplasm from cell to cell was not observed in the present study, possibly because of the slow rate of flow or because of the difficulty of examining the mycelium without disturbing it.

Introduction

The presence of perforations in the septa of the conidiophores of *Erysiphe Polygoni* DC., and the continuity of the protoplasm through the perforations were reported recently in a paper by Brodie and Neufeld (1). In the young conidiophore of that fungus, the transverse wall appears as a ring of shiny material which grows toward the inside until a disk-like septum is formed. In the centre of the disk, a hole is left through which passes a fine thread of protoplasm. There is, therefore, continuity of protoplasm from the base of the conidiophore to the apex. Pores and protoplasmic threads were also observed in the mycelium.

Special attention was not given to this matter by Brodie and Neufeld, inasmuch as it was felt that Buller's treatment (2) of the subject of protoplasmic continuity in fungi had established the essential facts. Demonstration of septal pores in the Erysiphaceae merely added one more group to a long list covering most of the groups of fungi[3].

However, subsequent studies of the conidiophores and mycelium of *Erysiphe graminis* DC. have shown that this fungus, like *E. Polygoni*, is a favourable subject for the demonstration of septal pores and protoplasmic continuity. By the use of a slight modification of Wahrlich's (6) technique (to be described in detail later in this paper), it was found possible to obtain excellent preparations showing the continuity of protoplasm, through septal pores, from cell to cell in chains of conidia and from cell to cell in the mycelium.

The purpose of this paper is to establish the existence of septal pores and protoplasmic continuity in *Erysiphe graminis* by means of photomicrographs

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[3] *Pores in the septa of mycelium of Sphaerotheca Castagnei Lev. (Erysiphe Cichoracearum DC.) were observed by P. A. Dangeard in 1897 (Botaniste (Sér. 5), 5 : 255. 1897).*

and drawings. This has seemed especially desirable because the question as to whether septal pores and protoplasmic bridges actually exist in the higher fungi has been raised by such workers as Noble (4, 5) who reported being unable to find pores in the septa of the mycelium of *Typhula Trifolii* Rostr.

Material and Methods

The fungus used in this investigation was *Erysiphe graminis* DC., which appeared on barley plants in the greenhouses of the University of Manitoba in December, 1941. It was transferred to the variety of barley, O.A.C. 21, and cultures were maintained on young barley plants inoculated by shaking spores from an older culture on to seedlings 3 in. high. Infected plants were kept on the greenhouse bench in full light. The fungus was usually at its best for study about a week or 10 days after inoculation.

Wahrlich's technique for demonstrating septal pores and protoplasmic continuity has been described by Buller (2, pp. 89–91). It consists of fixing the living fungus in a watery solution of iodine in potassium iodide, treating with chlor-zinc iodine to cause the walls of the fungus to swell, and staining with a strong solution of iodine in potassium iodide.

Difficulty was experienced at first in obtaining good fixation. Shrinkage caused the thin thread of protoplasm connecting adjoining cells to break. After many trials, the most satisfactory concentration for good fixation was found to be that made by diluting the strong iodine solution about 1 : 20. The exact proportions used are given herewith.

Strong iodine in potassium iodide solution:

Iodine	3 gm.
Potassium iodide	3 gm.
Distilled water	20 cc.

This strong stock solution was used: (1) for making the dilute fixative and (2) for staining the protoplasm.

The dilute fixative was made by adding 10 drops of the strong iodine in potassium iodide solution to 20 cc. of distilled water.

The complete schedule follows:

1. Fix fresh material in *diluted* iodine in potassium iodide for two hours.
2. Rinse with distilled water.
3. Add chlor-zinc iodine to material on slide. (It may be necessary to heat cautiously until steam appears.)
4. Remove chlor-zinc iodine with blotting paper and rinse with water.
5. Add several drops of strong iodine in potassium iodide and allow to stand 30 min.
6. Remove strong solution and rinse quickly with dilute solution.
7. Mount in chlor-zinc iodine, or in 30% glycerine.

Nigrosin was also used as a stain (10% aqueous) and, although it did not reveal any more detail than did the iodine stain, it was found more satisfactory in making preparations to be photographed.

General Appearance of Preparations

Using the method described above, the mycelium, conidiophores, and conidia of *Erysiphe graminis* presented the following appearance.

The lateral walls and septa were light yellow, highly refractive, and swollen. The septa appeared more swollen than the lateral walls and often became six to eight times their original thickness (Fig. 7). The extent to which the walls were swollen varied considerably in different preparations. Prolonged heating would cause great swelling but did not improve the appearance of protoplasmic connections.

The degree of plasmolysis of the protoplasts varied from very slight (Figs. 10, 11) to severe (Figs. 12, 13). Where plasmolysis had been severe, the protoplasmic connection between cells was usually broken (Fig. 8*a*).

When strong iodine in potassium iodide was used as the stain, the protoplasts were stained deep brown, almost black. Within them, various granules could be seen, and frequently a conidium was found containing a single stained nucleus (Fig. 11). Staining of the nuclei was more common when nigrosin was used (Fig. 6).

In none of the preparations was it possible to discern the double nature of the wall suggested by the drawings from Wahrlich's paper that were reproduced by Buller (2, pp. 90 and 94).

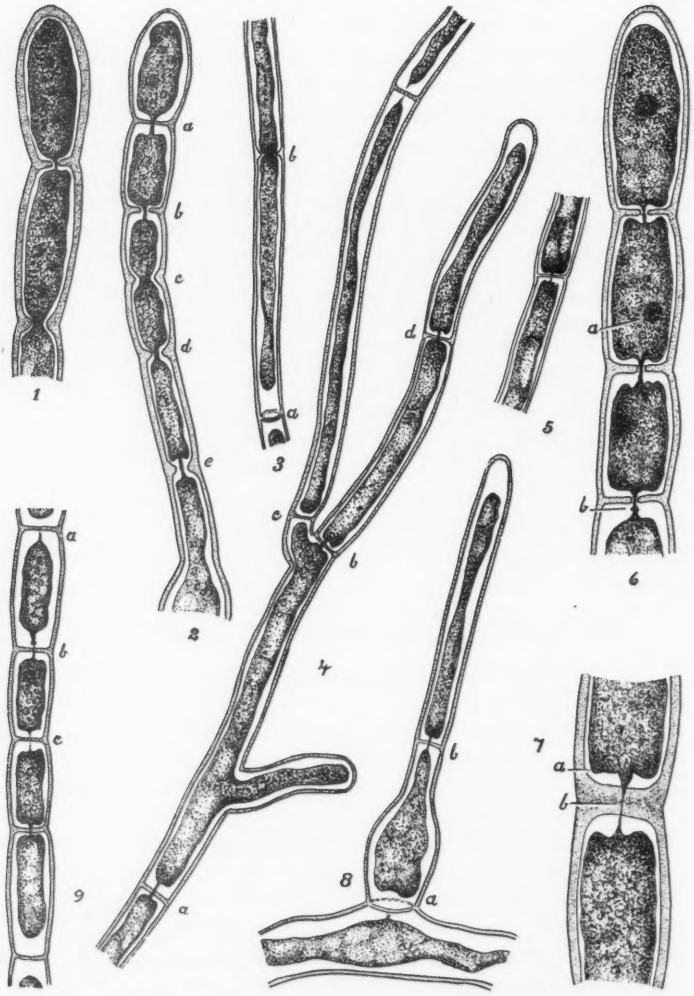
The Septal Pore and Protoplasmic Connections

In *Erysiphe graminis*, the protoplasts in living, actively growing mycelial cells and conidial cells in chains are connected by thin strands of protoplasm. When the septum between two cells is young, the protoplast is but slightly constricted by the septum (Fig. 2*c* and Fig. 3*b*); but, as the ring of wall material gradually closes, the protoplasmic connection between cells is reduced to a very thin thread of cytoplasm between 1 and 1.5 μ thick. Sometimes the thread appears homogeneous, at other times granular. Frequently there is a knot on the cytoplasmic strand (Fig. 11, *a*, *b*, and Fig. 6*b*). Even where severe plasmolysis has caused the thread to be broken, traces of it can frequently be seen at the ends of the protoplasts as shown in Fig. 9, *a*, *c*.

Connections are present between mycelial cells everywhere in the mycelium, from growing hyphal tips (Fig. 4*d*) back into older mycelium (Fig. 5). They are harder to demonstrate in older mycelium, however, where they frequently become broken between highly vacuolate cells.

In *Erysiphe graminis*, each chain of conidia arises from a large bulbous basal cell. The protoplasmic connection between this cell and the mycelium below it is usually very conspicuous, the thread being long. This connection is illustrated in the photomicrograph, Fig. 14.

It was surprising to find that protoplasmic connections are almost invariably present between adjacent conidia in chains. Often a certain conidium in a chain (e.g. *b* in Fig. 15) would appear to lack connection with its fellows, but



Erysiphe graminis DC. *All figures except Fig. 6 are from material stained with iodine.*

FIG. 1. *Apex of chain of conidia, showing much swollen walls and protoplasmic connections between spores.* ×*1000.* FIG. 2. *Chain of conidia showing protoplasmic continuity from bulbous basal cell to terminal conidium:* a, *transverse septum searcely swollen;* b *to* e, *transverse septa swollen in varying degrees.* ×*830.* FIG. 3. *Hypha near growing tip, showing fully formed septum at* a *(the pore not evident) and protoplasm severed by extreme contraction, and young septum at* b. ×*645.* FIG. 4. *Mycelium showing protoplasmic connections at* a, b, *and* d; *protoplasmic continuity disrupted at* c *by contraction.* ×*830.* FIG. 5. *Distinct protoplasmic thread between two cells in old part of mycelium.* ×*830.* FIG. 6. *Chain of conidia stained with nigrosin showing protoplasmic connections, nucleus at* a, *and knot on protoplasmic thread at* b. ×*1200.* FIG. 7. *Connection between two conidia at* b, *and lobed appearance of end of protoplast at* a. ×*1330.* FIG. 8. *Young conidiophore before spore formation; at* a, *protoplasmic connection with bulbous basal cell broken by contraction; at* b, *connection clearly visible.* ×*830.* FIG. 9. *Chain of conidia showing protoplasmic threads: at* a *and* c, *threads partly ruptured by contraction.* ×*830.*

always connections were found between other conidia in the same chain. The apparent lack of protoplasmic connections in these examples is probably to be explained by shrinkage due to imperfect fixation, with consequent rupture of the delicate thread connection.

In *Erysiphe Polygoni*, protoplasmic continuity is broken between the terminal mature conidium and the conidiophore, and the pore in the septum is plugged some time before the spore is shed (1). No evidence of plugging of the septum of the terminal conidium in *E. graminis* has been found in the course of the present investigation. Protoplasmic continuity is maintained even into the terminal conidium (e.g. Figs. 1, 2, 6).

The pore in the septum has only rarely been seen directly, although its presence is indicated beyond all doubt by the continuity of protoplasm through the septum. In a few preparations, the pore was seen directly, usually in hyphae or conidiophores that lay at such an angle that the microscope looked down on the face of the septum rather than on the edge. The photograph, Fig. 12, was made from a hypha in which a pore was particularly clearly seen at *a*. The hypha lay at a slight angle and, although the photograph leaves much to be desired, the pore in the centre of the septum is fairly well shown. The septum was near the growing tip of a hypha, and the pore is therefore larger than is found in fully formed septa.

Slight variations in optical conditions made a great difference to the appearance of the septum in preparations. Usually it appeared as a relatively thick unbroken line (Fig. 4). Frequently, however, it appeared broken (Fig. 2), especially where the walls had been greatly swollen. Whether the septum had the *appearance* of being broken or not, the protoplasmic thread was always continuous from one protoplast to the next, unless it had itself been broken by shrinkage. There does not seem to be any room for argument that because protoplasts were occasionally seen with no protoplasmic bridge between them (because of imperfect fixation), there is no pore in the septum. In fact, pores were occasionally seen even where no protoplasmic connection could be found.

A peculiar feature of the stained preparations which may be noted is the lobed appearance of the protoplasts adjacent to the septa (Fig. 7 and Fig. 15, *a*, *b*). The same feature can be seen in Wahrlich's illustrations reproduced by Buller (2, p. 90).

The photographs reproduced in Plate I were all taken with an oil immersion lens (Zeiss, N.A. 1.3) which had a shallow focus. It was therefore difficult to photograph protoplasmic threads that lay at different depths in a chain of cells. For example, the apparent lack of protoplasmic threads at *a* in Fig. 16, and at *b* in Fig. 15, is due solely to the threads being quite out of focus.

Although it may be thought that a treatment of delicate protoplasmic structures that involves heating with chlor-zinc iodine might produce artifacts, the fact is that it is possible to see the protoplasmic connections which have been described in *untreated* mycelium and conidial chains of *Erysiphe graminis*.

Several times, *living* mycelium and conidial chains were examined when protoplasmic bridges between conidia in chains were seen clearly with the oil immersion lens. There can therefore be no doubt about the existence of protoplasmic continuity between cells in this fungus.

As a warning to those who may wish to employ Wahrlich's technique for the demonstration of protoplasmic threads and septal pores in other fungi, it may be well to re-emphasize the necessity of obtaining good fixation. Fixatives other than that used in the present investigation might prove more satisfactory. It is essential that no marked contraction of the protoplasm take place in order that the delicate strands that pass through the pores remain intact. At the outset, the writer had to find by trial the dilution of iodine fixative that gave the best results, and the conditions for fixation and staining may be expected to be slightly different in different fungi.

Search for Protoplasmic Streaming

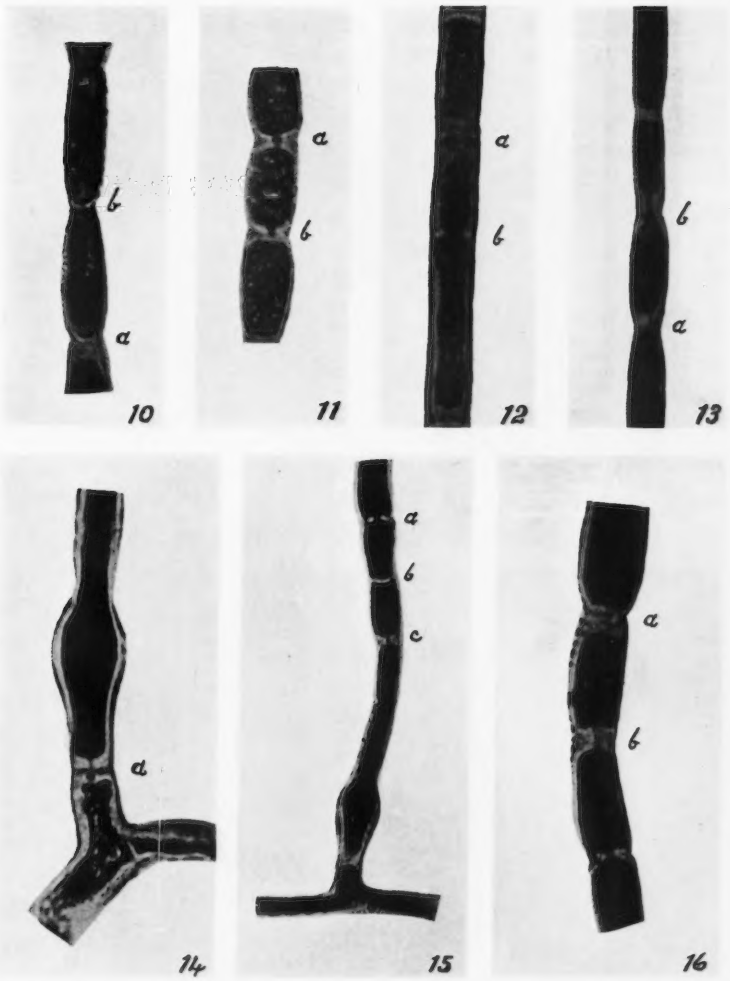
It was pointed out by Brodie and Neufeld (1) that septal pores and protoplasmic continuity are very important in the Erysiphaceae. Since the haustoria absorb the food material, and since the mycelium and condiophores are aerial, food materials and protoplasm must pass from the haustoria to the aerial conidiophores via the pores in the transverse septa of the mycelium.

The streaming of cytoplasm from cell to cell has been demonstrated for a number of fungi by Buller (2), Dowding (3), and by others, and might be expected in the mycelium and condiophores of *E. graminis*. Brodie and Neufeld (1) reported being unable to observe any flow of cytoplasm in living mycelium of *E. Polygoni*, and the writer therefore made a special effort to observe cytoplasmic streaming in *E. graminis*.

Patient and exhaustive study of all parts of the mycelium and conidiophores failed to reveal any continuous movement of cytoplasm of living material of *E. graminis*. At first, mycelium was carefully removed from infected leaves with needles and mounted in water for examination. Later, in order to avoid (as far as possible) injury to the mycelium, pieces of barley leaves infected with mildew were gently pulled apart lengthwise with the purpose of exposing, between the long strips of leaf, bits of mycelium that had not been greatly disturbed. In some examinations, the mycelium was mounted in liquid petrolatum instead of water. The cytoplasm could be clearly seen in all preparations. It is very granular and it should be possible to see streaming if it takes place.

Occasionally a slight movement was observed, but this was brought about by rupture of a hypha or by a sudden shift in the position of vacuoles. At no time was cytoplasmic streaming observed, although the search was carried on for several weeks in material of various ages.

It is most improbable that no streaming of cytoplasm at all occurs in the mildew mycelium. The mycelium grows very slowly, and failure to observe streaming by direct observation may be due to the slow rate at which it takes



EXPLANATION OF PLATE

Erysiphe graminis DC. Photomicrographs of material stained in nigrosin, except Figs. 10, 11, and 12 which are of material stained in iodine. All photographs untouched except for blocking out of background.

FIG. 10. *Two conidia near end of chain showing connection at* b*; connection at* a *broken by shrinkage.* ×*660.* FIG. 11. *Three conidia showing connections at* a *and* b. ×*830.* FIG. 12. *A bit of mycelium near growing end showing a connection at* b*; at* a*, a septum is seen thrown slightly out of focus to show the pore in the centre.* ×*660.* FIG. 13. *Chain of conidia with connecting threads at* a *and* b. ×*580.* FIG. 14. *Connection between basal cell of chain of conidia and mycelium below, shown at* a. ×*830.* FIG. 15. *Conidia with connections at* a *and* c*; at* b*, the connection was present but completely out of focus.* ×*410.* FIG. 16. *Chain of conidia showing protoplasmic bridge at* b*; a thread was also visible at* a *in the material but it is not in focus in the photograph.* ×*830.*

place. It may also be due to the difficulty of examining the mycelium without disturbing it.

Acknowledgment

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A STAINING TECHNIQUE FOR EVALUATING THE TOXICITY OF AN ANTIBIOTIC SUBSTANCE OF MICROBIOLOGICAL ORIGIN[1]

By H. Katznelson[2]

Abstract

A method is described in which the absorption of neutral red by dead cells of *Schizosaccharomyces pombe* is used as an index of the toxicity of an antibiotic agent produced by a bacillus.

Progress in research work with antibiotic substances produced by microorganisms is frequently delayed because a rapid method for determining the toxicity of these agents is lacking. The most common procedures for evaluating the activity of these substances utilize their ability to inhibit, dissolve, or kill the test organism (1, 4, 5, 7). These techniques often involve standardization of bacterial suspensions, plating, incubation for a period of 24 or 48 hr., and counting of plates; a study of the influence of these toxic principles on fungi not infrequently involves incubation for two to seven days before satisfactory results are obtained (2). It is obvious that a procedure that will eliminate such a step as a long incubation period alone, and yield results within two or three hours is highly desirable.

It was recently reported (2) that the yeast *Schizosaccharomyces pombe* was one of many fungi inhibited (and killed) by a thermostable substance elaborated by an aerobic sporeforming bacillus. This yeast was found by Knaysi (3) to take up neutral red on dying; the cells usually stained a deep red but even faintly stained cells were shown to be dead whereas living, healthy cells remained colourless. The present paper reports an attempt to utilize this phenomenon in various experiments with the toxic agent.

Description of the Method

The yeast was grown on nutrient agar containing 1% peptone and 2% dextrose for 24 hr. at 28° C. after which the cells were suspended in potato dextrose broth at pH 6.3; the suspension was standardized by means of a counting chamber so that one ml. contained 160,000,000 cells. To one ml. of the toxic test material in a small glass tube ($2\frac{1}{2} \times \frac{3}{8}$ in.) were added 0.5 ml. of the cell suspension and 0.5 ml. of a 1 : 5000 aqueous solution of neutral red; the tube was plugged, shaken thoroughly, placed in a water-bath maintained at 36° C. for three hours, and agitated intermittently. On removal from the water-bath the tube was again shaken and some of its contents transferred to a counting chamber. Counts were made of the total number

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Contribution from the Department of Bacteriology, Cornell University, Ithaca, N.Y., and the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa. (Journal Series No. 158.)

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and the number of stained, shrunken organisms in 20 of the smallest squares and the results were expressed as percent stained cells. When quantitative data were not required the procedure was expedited by placing a drop of the suspension in the test-tube on a slide, examining it under the microscope, and estimating roughly the percentage of stained cells in several fields. The accompanying photographs (Fig. 1) indicate that differences between stained and unstained cells are readily apparent, a fact that facilitates the quantitative or semiquantitative determination of the toxicity of the antibiotic substance.

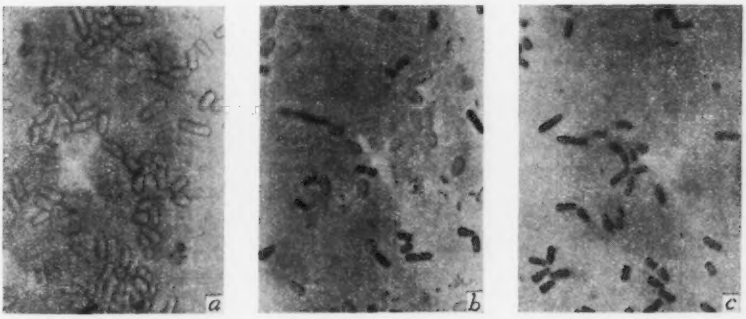

FIG. 1. *Influence of toxic medium on S. pombe as indicated by neutral red staining; (a) check; (b) 50% toxic medium; (c) 100% toxic medium. (×500).*

The final concentration of neutral red in the incubating mixture is 1 : 20,000 and is not harmful to the yeast. The reaction of the test and suspending fluids should be slightly acid (pH 6.0 to 6.5) as neutral red becomes yellowish and useless in an alkaline medium. Various suspending solutions (buffers) for the yeast cells were tested and potato dextrose peptone broth was selected (Table I).

The graphs in Fig. 2 are illustrative of the results of many experiments to determine the conditions that would favour the greatest number of cells being

TABLE I

STAINING OF *Schizosaccharomyces pombe* IN THE PRESENCE OF A TOXIC AGENT AS AFFECTED BY DIFFERENT BUFFER SUSPENDING SOLUTIONS

	Suspending fluids, pH 6.3					
	Borate	Citrate	Glycine	Phosphate	Phthalate	Potato dextrose broth
	Stained cells, %					
Controls	1.0	20.0	1.0	0.1	0.1	0.2
Toxic substance added	27.0	35.0	25.0	34.0	21.0	89.0

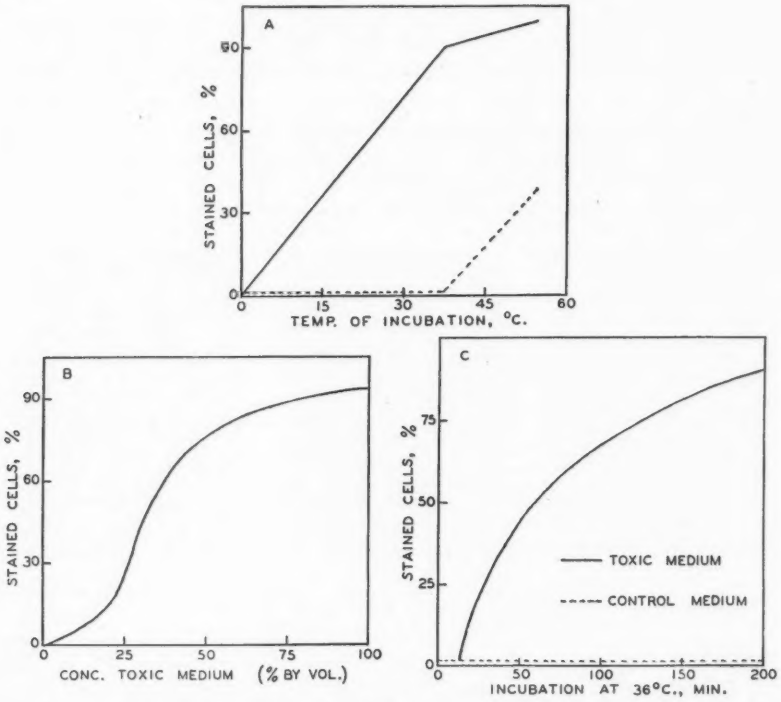

FIG. 2. *Influence of temperature of incubation, concentration of toxic medium, and period of incubation on the staining of Schizosaccharomyces pombe by neutral red.*

killed by the agent. In the trials represented by Graphs A and C, undiluted toxic medium (2) was used. A temperature of 36° C. and an incubation period of three hours were finally selected (a considerable number of cells were killed in the control series at 55° C.). Graph B indicates the effect of different concentrations of toxic medium on the yeast. The most effective range appears to be between 15 and 50% (by volume) of toxic medium.

In the numerous studies with the toxic principle the above technique proved a rapid, useful tool. Concurrently, however, the fungus *Rhizoctonia Solani* was also used as an index of the potency of the inhibiting substance for comparative purposes. The following experiments illustrate the applicability of this staining procedure.

As previously reported (2) toxic medium is thermostable in neutral or slightly acid solutions, loses its potency when heated in a strongly alkaline medium, but retains it to some extent in strongly acid solutions. In these tests, toxic and potato dextrose media were adjusted to pH < 1 with hydrochloric acid and pH > 12 with sodium hydroxide and heated 15 min. in boiling water; tubes with the above media kept at pH 6.0 were also included in this series.

After heating, the pH of all the tubes was brought to 6.0 and all fluids including unheated toxic and potato dextrose media were tested by the yeast staining method and by the dilution method with *R. Solani*; the results are presented in Table II. Both methods bear out the observation regarding the influence of heating toxic medium in acid and alkaline solutions.

TABLE II

INFLUENCE OF HEAT ON TOXIC MEDIUM IN ACID AND ALKALINE SOLUTIONS AS DETERMINED BY YEAST STAINING AND INHIBITION OF *Rhizoctonia Solani*

Test method		Treatment of medium							
		Unheated		Heated					
				pH < 1		pH > 12		pH 6.0	
		T.M.†	P.D.B.††	T.M.	P.D.B.	T.M.	P.D.B.	T.M.	P.D.B.
Yeast staining*		90	2	39	1	1	1	92	1
Inhibition of *R. Solani***	Conc. of test material (% by vol.)								
	0	4	4	4	4	4	4	4	4
	1	4	4	4	4	4	4	4	4
	3	2	4	4	4	4	4	2	4
	5	1	4	4	4	4	4	0	4
	7.5	0	4	3	4	4	4	0	4
	10	0	4	2	4	4	4	0	4
	15	0	4	0	4	4	4	0	4
	25	0	4	0	4	4	4	0	4
	50	0	4	0	4	1	1	0	4

* *% Stained cells.*
** *Good growth = 4; no growth = 0.*
† *T.M. = toxic medium.*
†† *P.D.B. = potato dextrose broth.*

In an experiment to determine the influence of pH of medium on the production of toxic principle by the bacillus a range from pH 4.4 to 7.5 was used. Flasks at different pH levels were inoculated and incubated for seven days after which the pH was adjusted to 6.0, the medium diluted with two volumes of potato dextrose broth and tests made using the yeast technique. Apparently an acid reaction favours the production of the inhibitory agent (Table III).

TABLE III

ELABORATION OF TOXIC MATERIAL AT VARIOUS pH LEVELS

pH of medium	4.4	4.8	5.5	5.9	6.2	6.7	7.1	7.5
Stained yeast cells, %	59	60	35	36	35	21	20	13

Agitation of the culture medium is frequently employed to stimulate the activity of certain organisms (6) but it was deleterious in this instance (Table IV).

TABLE IV

EFFECT OF AGITATION OF CULTURE MEDIUM ON PRODUCTION OF TOXIC MATERIAL AS DEMONSTRATED BY YEAST STAINING

Concentration of medium tested (% by volume)	Treatment of medium			
	Continuous agitation for 1 week		No agitation	
	Culture medium	P.D.B. control	Culture medium	P.D.B. control
	Stained cells, %			
0	0	0	0	0
25	0	0	35	0
50	0	0	60	0
100	0	0	100	0

The composition of the culture medium exerts a decided influence on the elaboration of inhibitory material. With potato dextrose broth as base, varying amounts of peptone were added and the resulting culture media were tested with both methods (yeast staining and inhibition of *R. Solani*). Both procedures indicate that low concentrations of peptone (resulting in poor growth of the bacillus) are unfavourable for the production of the toxic substance (Table V).

TABLE V

STAINING OF *S. pombe* AND INHIBITION OF *R. Solani* BY TOXIC MATERIAL PRODUCED IN POTATO DEXTROSE BROTH CONTAINING VARIOUS AMOUNTS OF PEPTONE

Test method		Peptone, %						
		0.05	0.1	0.5	1.0	1.5	2.0	2.5
Yeast staining, %		10	15	53	74	86	88	90
Inhibition of *R. Solani**	Conc. test material (% by volume)							
	0	4	4	4	4	4	4	4
	1	4	4	3	3	3	3	3
	3	3	2	1	0	0	0	0
	5	2	2	1	0	0	0	0
	7.5	2	1	0	0	0	0	0
	10	1	1	0	0	0	0	0
	15	1	0	0	0	0	0	0

* *Good growth = 4; no growth = 0.*

By similar methods it was found that low concentrations of dextrose or sucrose were also unfavourable, the optimum concentration being 2.5%. Sucrose was slightly less favourable than dextrose (Table VI). The same trend was observed with the *R. Solani* technique.

TABLE VI

EFFECT OF CONCENTRATION OF GLUCOSE AND SUCROSE ON YIELD OF TOXIC MATERIAL

Sugar used	Sugar concentration, %					
	0.1	0.5	1.0	1.5	2.0	2.5
	Stained cells, %					
Dextrose	30	60	60	68	81	92
Sucrose	32	49	58	57	60	72

Charcoal was found to remove the toxic substance from the culture medium (2). Again the yeast staining and *R. Solani* methods bear this out (Table VII).

TABLE VII

ADSORPTION OF TOXIC MATERIAL BY CHARCOAL

Test method		Treatment of material			
		Toxic medium		Potato dextrose broth	
		Untreated	Charcoal treated	Untreated	Charcoal treated
Yeast staining, %		90	1	1	1
Inhibition of *R. Solani**	Conc. test material (% by volume)				
	0	4	4	4	4
	1	1	4	4	4
	2	1	4	4	4
	3	0	4	4	4
	5	0	4	4	4
	10	0	4	4	4
	25	0	4	4	4
	50	0	4	4	4
	75	0	4	4	4
	100	0	4	4	4

* *Good growth = 4; no growth = 0.*

The above experiments suffice to indicate the usefulness of the yeast staining technique for studying the lethal nature of an antibiotic agent of microbiological origin. It may also find application in evaluating the fungicidal or bactericidal property of a wide variety of commercial disinfectants and chemotherapeutic agents.

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INDEX TO VOLUME 20

Authors

SECTION C

INDEX TO VOLUME 20

Subjects

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MAIN ARTERIES IN THE REGION OF THE NECK AND THORAX OF THE AUSTRALIAN CASSOWARY[1]

BY FRED H. GLENNY[2]

Abstract

A mature specimen of the Australian cassowary (*Casuarius australis* Wallace) was dissected and a diagram of the main arteries in the neck region prepared. In the arrangement and distribution of these arteries, the cassowary differs from other species of birds previously studied. The left radix aortae and left ductus Botalli remain as a ligamentous vestige, the ligamentum aortae. The left internal carotid artery alone enters the hypapophysial canal. The right internal carotid artery is lacking or has become modified to form the ascending oesophageal artery of the adult, in the basal portion of the neck. The pattern of the arrangement of the main arteries in the neck and thorax shows a relatively primitive avian condition.

In several recent papers, the writer (4 to 8) has been able to demonstrate a large variety of arrangement patterns for the main arteries in the neck and thorax of birds of various orders and families. At the same time it has been possible to add in small part to the present knowledge of the gross anatomy of this class of vertebrates. The present paper deals with the distribution of the main neck and thoracic arteries and their branches in the cassowary (*Casuarius australis*).

In some minor respects, the arrangement of the main arteries in the neck and thorax resembles that found in the kiwi (*Apteryx australis mantelli*) but owing to the much greater size of the cassowary, a greater number of vessels are present to supply the various areas. The origin and distribution of some of the more important arteries of the neck differ in the two groups to such an extent that they may be regarded as having fundamental ordinal characters.

Huxley (9), in 1867, placed the cassowaries and kiwis in the same order, the Ratitae, but at the same time he recognized certain fundamental structural differences between the two groups. Later, however, Wetmore (11) divided the paleognathous birds into several groups and accorded ordinal rank to each.

In 1873, Garrod (3) reported on the carotid arteries of birds and remarked that in the Struthiones, both carotid arteries (left and right) were present in *Struthio camelus* Linné, *Casuarius bennetti* Gould, *Casuarius bicarunculatus* Sclater, and *Dromaeus novae-hollandiae* (Latham), whereas the left alone was

[1] *Manuscript received July 23, 1942.*
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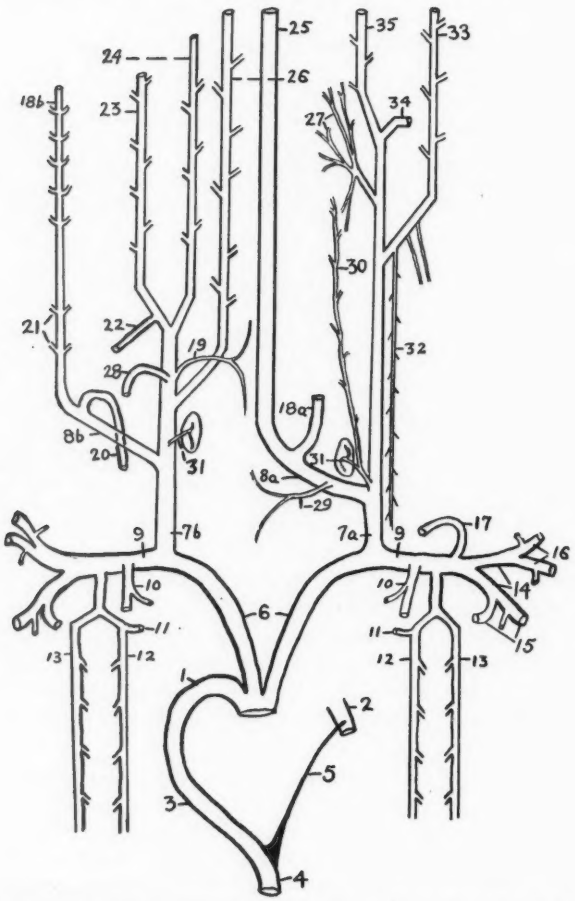

FIG. 1. *Diagrammatic representation of the main arteries in the neck and thorax of the Australian cassowary (Casuarius australis). Ventral view.*

KEY TO ABBREVIATIONS

(*1*) Right systemic (fourth aortic) arch; (*2*) left pulmonary (sixth aortic) arch; (*3*) right radix aortae; (*4*) dorsal aorta; (*5*) ligamentum aortae; (*6*) innominate arteries; (*7*) carotid (third aortic) arch; (*8a*) base of left internal carotid artery; (*8b*) cervico-intercostal artery; (*9*) subclavian arteries; (*10*) coracoid major; (*11*) coracoid minor; (*12*) ventral intercostal; (*13*) lateral intercostal; (*14*) pectoral; (*15*) coracoid and pectoral branches of pectoral artery; (*16*) axillary and pectoral branches of pectoral artery; (*17*) left basicervical; (*18a*) left vertebral; (*18b*) right vertebral; (*19*) right tracheal; (*20*) posterior dorsal intercostal; (*21*) anterior dorsal intercostals; (*22*) right subscapular; (*23*) right lateral superficial cervical; (*24*) right lymphatico-cutaneous; (*25*) left internal carotid (in hypapophysial canal); (*26*) ascending oesophageal; (*27*) myocervical; (*28*) right basicervical; (*29*) left tracheal; (*30*) thyroid-lymphatic; (*31*) thyroid gland and artery; (*32*) left posterior superficial cervical; (*33*) left anterior superficial cervical; (*34*) left ventral cutaneous; (*35*) left ventral anterior superficial cervical.

present in *Rhea americana* Linné, *Apteryx (australis) mantelli* Bartlett, and *Apteryx owenii* Gould. With regard to the arrangement of the carotids in the latter three species, the present writer's observations on the kiwi (*7*) and rhea are in agreement with the reported findings of Garrod (*3*), but the specimen of *Casuarius australis* which was examined by the present writer showed the left carotid artery alone as the vessel that entered the hypapophysial canal.

The following observations serve as an explanation of the diagram (Fig. 1) and the general distribution of the arteries of the neck and thorax.

Observations

In *Casuarius australis* the distal portion of the left embryonic sixth aortic arch remains as a ligament of fusion with the ligamentous vestige of the left radix aortae, to form the ligamentum aortae (*5*), while the corresponding portion of the right embryonic sixth aortic arch completely disappears in the mature bird. This probably results from atrophy of the right ligamentum Botalli and its ultimate fusion with the pulmonary artery proximally and the right radix aortae (*3*) distally.

As in other species of birds, the innominate arteries (*6*) give rise to the left (*7a*) and right (*7b*) carotid arches and the subclavian arteries (*9*). Each of the latter then gives rise to four arteries, the coracoid major (*10*), the intercostal (*12* and *13*), and two pectoral arteries (*14*). The intercostal artery divides to form ventral (*12*) and lateral (*13*) branches, and a small branch of the ventral intercostal serves as a coracoid minor (*11*) artery. The pectoral arteries are modified to supply the wings, pectoral muscles, and coracoid and scapular areas (*15* and *16*). In addition, the left subclavian artery sends off a basicervical artery (*17*) to supply the tissues at the base of the neck.

Anteriorly, each carotid arch (*7*) divides to give rise to a superior branch and an inferior branch. The left inferior artery gives rise to a small tracheal artery (*29*) before it divides to form the deep vertebral artery (*18a*) and the internal carotid artery (*25*) which enters the hypapophysial canal in the median line of the neck. The left superior artery sends branches to the thyroid gland (*31*) and connective tissues at the base of the neck (*30*), then bifurcates to form the ventral superficial (*35*) and lateral superficial (*33*) cervical arteries. The former sends off a branched myocervical artery (*27*), a ventral cutaneous artery (*34*), and several branches to the lymphatic glands and connective tissues along the left side of the neck. The lateral superficial cervical (*33*) gives rise to a posterior superficial cervical artery (*32*).

The superior branch of the right carotid arch gives rise to the posterior dorsal intercostal artery (*20*), several anterior intercostal arteries (*21*), and then becomes the right vertebral artery (*18b*). The inferior branch gives rise to the thyroid arteries (*31*) before sending off the large ascending oesophageal artery (*26*), right tracheal artery (*19*), right basicervical artery (*28*), lateral superficial cervical artery (*23*), and lymphatico-cutaneous artery (*24*). The

lateral superficial cervical gives off a small subscapular branch (*22*) before sending several branches to the muscles of the neck and surrounding connective tissues. The lymphatico-cutaneous artery supplies the lymphatic glands on the right side of the neck and ultimately terminates in the skin of the neck.

Discussion

It will be noted that, in the cassowary, the skin of the neck is supplied by vessels which arise in the cervical region, whereas in the kiwi, the skin is supplied by vessels which arise from the subclavian and pectoral arteries. Furthermore, the kiwi retains both the left ligamentum aortae and the right ligamentum Botalli (8), while the cassowary retains the ligamentum aortae alone.

Although these two species of birds differ in many respects, they do show two points of similarity. First, the left internal carotid artery alone enters the hypapophysial canal to pass forward to the head. Secondly, the axillary and pectoral arteries show remarkable adaptation to the reduction and modification of the wings and pectoral muscles in both species.

In general, however, the arterial arrangement in the cassowary is quite singular and largely unlike that observed in other species of birds which have thus far been examined by the writer. Representatives of more than 12 orders of birds have been studied, in part, to date, although but a few families have been reported (4 to 8). References to these arteries and their vestiges have been made also by Beddard (1), Bhaduri (2), and Stresemann (10).

Acknowledgments

The writer is indebted to Meems Bros. and Ward, Oceanside, Long Island, N.Y., for their kindness in making available the specimen used in this study, and to Dr. E. Horne Craigie, Department of Zoology, University of Toronto, Toronto, Ont., for his suggestions in the preparation of this paper.

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POLARIZATION AND PROGRESSION IN PAIRING

III. PACHYTENE OBSERVATIONS IN *NEODIPRION* (HYMENOPTERA)[1]

By Stanley G. Smith[2]

Abstract

From an analysis of 72 nuclei at pachytene in three species of *Neodiprion* it is shown (1) that the rate of pairing is independent of chromosome size, (2) the nucleolar chromosomes are at no disadvantage in pairing, and (3) the incompletions observed are the result of an interruption by fixation of the process of zygotene pairing. It is concluded that there is no fixed relationship between the intimacy of telophase association and pachytene pairing, the degree of relational coiling at diplotene, and the distribution of chiasmata at metaphase.

On the basis of the positions of interlocks and the size of the loops containing them, Smith and Boothroyd (36) have presented evidence that pairing in *Trillium erectum* pollen mother cells starts in the region of the centromeres and progresses outwards towards the ends of the arms of the chromosomes. Following Bennett (2), Frankel (14) had reached a similar conclusion from an analysis of configuration types occurring in various *Fritillaria* species with and without localization of chiasmata. In addition, however, he found evidence of a secondary, distal, contact point which, since it acts later, is held subject to the action of a time limit. Similarly, Darlington (7) holds that procentric pairing is primarily operative in both diploid and tetraploid species of *Paris* having localized chiasmata and that secondary contact points are situated in other regions of the chromosomes. The exact position in which pairing begins is considered to depend on the shape and size of the chromosome. Barber (1) finds in *Uvularia* that the reduction in chiasma frequency, induced by cold treatment, is differential in spatial distribution, the middle regions of long arms being more readily affected. He concludes that procentric and proterminal contact points are normally operative.

Darlington considers that his evidence implies a regular orientation of the chromosomes in the leptotene stage of the normal nucleus as the basis of regular co-ordination of pairing. Barber interprets his findings as showing that localization of pairing depends on (*a*) proximity of certain regions of the chromosomes, which is determined by a persistence of the telophase arrangement from the last premeiotic mitosis, and (*b*) a greater freedom of movement of short chromosomes or of the ends of long ones. Smith and Boothroyd hold that the centric and adjacent regions of homologues have an initial advantage in pairing as a result of the chromosomes being polarized since the last premeiotic anaphase disjunction, but recognized that the ends of the chromosomes, if not already associated, will also be brought together

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when the nuclear membrane is reformed, as is seen in a comparison of mitotic telophase and prophase (Rabl's (30) orientation). From a comparison of Diptera with other animals and plants, Smith (34) has given reasons for concluding that the pairing of homologues, consummated at pachytene, is initiated at the anaphase of the last premeiotic division.

Both Darlington and Barber assume that there is a time factor in pairing and that short chromosomes and short chromosome arms start pairing earlier than long ones and complete their pairing earlier. This they hold is the result of a greater freedom of movement in pairing *at zygotene*. Smith, on the other hand, concludes that short chromosomes and short chromosome arms can become more completely associated at the *last premeiotic telophase* as a direct result of their shortness and closer congression at the anaphase pole. Darlington's and Barber's assumption is based on interpretation of indirect evidence; certain data collected during a study of sawfly oocytes supply direct evidence on this point.

With further regard to the time factor as a limitation to pairing, Darlington (4, 5, 6) has stated that static bodies such as nucleoli delay the pairing of the chromosomes to which they are attached, and thus interfere with chiasma formation. He considers that this indicates why ". . . large nucleoli are as a rule situated near the ends . . ."; "Evidently they would hinder meiotic pairing too much if they lay in the middle of the chromosomes." This conclusion regarding interference is based on somewhat meagre evidence and is not supported by McClintock's (27) drawings of *Zea mays*, nor by Ernst's (8, 9) observations of pachytene in *Antirrhinum*. The analysis of the present data will go further in determining the general validity of this hypothesis. This is particularly important since the sex chromosome pair in many Diptera, including *Drosophila*, carries the nucleolus, and delay in pairing in localized regions will therefore affect the genetic map of the X-chromosome.

Finally, Darlington (4) reports for species of *Fritillaria* with a normal distribution of chiasmata that although "It has always been supposed that pairing in such species was complete . . .", he now rarely finds a nucleus with complete pairing. He attributes the earlier opinion to the fact that "An occasional unpaired segment would be taken as indicating that the nucleus was still in an active zygotene condition."

From the observations here presented it is intended to test the general applicability of three assumptions: (i) rapidity of pairing is dependent on the size of the units involved, (ii) nucleoli tether the chromosomes to which they are attached and thereby delay their pairing, and (iii) incomplete pairing of chromosomes is a characteristic even of species with a normal chiasma distribution.

Material

Females of three species of the hymenopteran genus *Neodiprion*, namely, *N. dubiosus*, *N. lecontei*, and *N. swainei*, have been studied at pachytene. Slides were prepared by the Feulgen squash method, after fixation for about

10 min. in a modification of Kahle's fluid (35). Light green was used to stain the nucleolus. All pachytene nuclei were drawn with the aid of a camera lucida using a Zeiss 1/12 homogene immersion objective and a 20× ocular; the single somatic metaphase illustrated was drawn using a Zeiss 1.5 mm., 1.3 N.A. objective.

Observations

The diploid number of chromosomes in females of all three species is 14. This is the number previously found by Smith (33) in *N. sertifer*. The centromeres are approximately median in all pairs of homologues except one of the three of medium size, so that in each of six pairs the arms are of almost equal length (see Fig. 1, the complement in a male of *N. lecontei*).

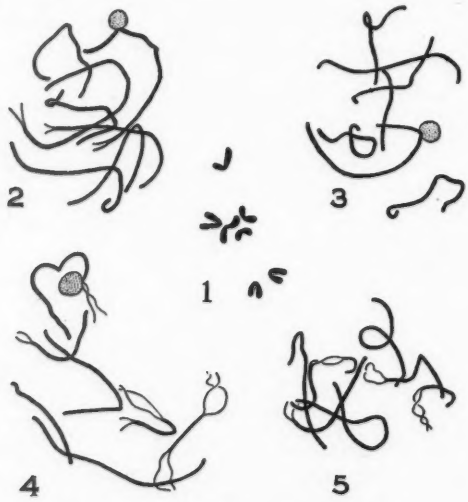

FIG. 1. *The somatic chromosomes of N. lecontei*, ♂. (×*2200*).
FIGS. 2 TO 5. *Pachytene chromosomes of females.* (×*1800*).
FIG. 2. *Polarization and distal incompletions in N. lecontei.*
FIG. 3. *Complete pairing in N. dubiosus.*
FIG. 4. *Distal incompletions in N. lecontei showing especially that of the nucleolar chromosome.*
FIG. 5. *Distal and one interstitial incompletion in N. swainei.*

As in other species of the Diprionidae (33), the arrangement of the seven bivalents at pachytene shows clear evidence of centric polarization (Fig. 2), although this is frequently more or less obscured by excessive pressure in making the preparation. In all a total of 72 whole complements have been drawn at pachytene from 17 females belonging to the three species (Table I). The association between the homologues, if complete, is then so close as to make the bivalents appear like single, thick threads (Fig. 3). The seven pairs are of different lengths. In Table I their individual lengths are expressed

TABLE I

THE DISTRIBUTION OF UNPAIRED REGIONS AMONG PACHYTENE BIVALENTS IN VARIOUS SIZE CLASSES

Species	No. ♀♀ examined	No. cells drawn	Length of bivalents expressed as percentage of whole complement							Total distals
N. lecontei	7	33	18.9 7*D**	17.3 7*D* + 1*I*	15.9 3*D*	14.7 2*D*	12.8 7*D**	11.3 10*D* + 1*I*	9.1 7*D*	41*D*
N. dubiosus	5	22	19.4 1*D*	17.2 —	15.9 3*D*	14.8 2*D*	13.4 2*D*	11.1 1*D*	8.2 2*D*	11*D*
N. swainei	5	17	19.5 —	17.1 —	15.7 —	14.6 2*D*	13.1 1*D*	11.3 1*D*	8.8 4*D*	8*D*
Mean length Total distals	17	72	19.3 7*D*	17.2 7*D*	15.8 6*D*	14.7 6*D*	13.1 9*D*	11.2 12*D*	8.7 13*D*	60*D*
Expected on random basis			8.6	8.6	8.6	8.6	8.6	8.6	8.6	$\chi^2 = 5.80$
Expected on length basis			11.6	10.3	9.5	8.8	7.9	6.7	5.2	$\chi^2 = 28.40$

*D = distal incompletion; I = interstitial incompletion; * = 1 distal interlock omitted from totals, see text.*

as percentages of the whole complement; they are arranged in decreasing order. In preparation the chromosomes were squashed flat so that there was very little or no optical foreshortening. The mean percentage lengths have been given in preference to absolute lengths because of variation between cells resulting from slight differences in stage and from the differential effect of the fixative.

Within the species the minimum length difference separating bivalents in the seven successive size classes was always at least 1.1 per cent, with the longest bivalent more than twice the length of the shortest. Between species the mean lengths of the bivalents compared in order of size never differed by more than 0.9 per cent. The three species therefore have complements sufficiently similar to warrant grouping them.

In 64 out of the total of 504 bivalents drawn and measured, pairing was incomplete (Figs. 2, 4, and 5) and in every case but two the incompletions were situated at the distal ends of the bivalents. Of these two interstitial incompletions, one was the result of "false" interlocking, its chromosomes having encircled those of another bivalent. The reason for the second (Fig. 5) could not be determined. Two of the distal incompletions were due to potential interlocking and therefore, like the previous two, will be omitted from further consideration. Thus there remains a total of 60 incompletions, 41 in *N. lecontei*, 11 in *N. dubiosus*, and 8 in *N. swainei*, to distribute between the seven size classes of bivalents.

Out of the total of 72 nuclei drawn, incomplete pairing occurred in only 30; one of these, in *N. lecontei*, was due to interlocking and may therefore, from

the present point of view, be considered as completely paired. Pairing was thus complete in 43 nuclei, 15 in *N. lecontei*, 16 in *N. dubiosus*, and 12 in *N. swainei*. Considering the frequency of incompletions in individual females, nine of the 17 examined had only cells with complete pairing, seven had cells with both complete and incomplete pairing and one had only partial pairing. In this single female, however, only two cells were suitable for detailed analysis. The occurrence in all individuals except one of cells in which the chromosomes are fully associated suggests that the observed incompletions were the result of fixing the ovaries while the nuclei were still undergoing active pairing. This is shown also (Table II) by the fact that as the number of incompletions per cell increases, from zero to six—the maximum number observed—the frequency of cells showing these numbers of incompletions steadily decreases from 43 to one. It might here be mentioned that in these 72 nuclei and in about three times as many more (which for various reasons were unsuitable for exact measurement) there was absolutely no evidence of structural hybridity.

TABLE II

THE FREQUENCY OF CELLS WITH DISTAL INCOMPLETIONS IN DIFFERENT SPECIES

Species		Number of incompletions per cell								
		0	1	2	3	4	5	6	7	Total
N. lecontei	No. of cells	15	8**	3	4*	1	1	1**	0	33
N. dubiosus	No. of cells	16	3	2	0	1	0	0	0	22
N. swainei	No. of cells	12	4	0	0	1	0	0	0	17
	Total	43	15	5	4	3	1	1	0	72

*, ** *Omitting one interstitial and one distal incompletion respectively, see text.*

In Table I the distribution of the unpaired ends among the various types of bivalents is given and compared by means of the χ^2 test of goodness of fit with expectation, first, on a random basis and, second, on the assumption that the time required for complete pairing is directly proportional to length. From the first comparison $\chi^2 = 5.80$, which, with $N = 6$, gives $P = 0.50$; from the second, $\chi^2 = 28.40$ and $P = < 0.01$. It is therefore evident that the short chromosomes in these species complete their pairing no more readily than the long chromosomes. Since, as already stated, all the chromosomes, with the exception of one in the three middle size classes, are mediocentric, a similar conclusion holds with regard to the chromosome arms.

In 26 of the 30 cells with partial pairing, the nucleolar chromosomes could be identified by their association with the nucleolus. The nucleolar constriction at mitotic metaphase is situated about half-way along an arm of either the longest or second longest chromosome (Fig. 1). In the 26 cells there was a total of 52 incompletions, 51 distal, and one interstitial, omitting

those resulting from interlocking. Of the unpaired regions, 34, including the single interstitial, occurred in the bivalents of cells in which the nucleolar chromosomes were completely associated, three were situated on this chromosome pair in cells in which the remaining chromosomes were fully paired, and five involved the nucleolar pair in cells having 10 other incompletions (see Fig. 4). In short, the nucleolar chromosomes were incompletely associated on eight occasions, the other chromosomes on 44.*

Discussion

Darlington (4) has assumed that the rarity of nuclei with complete pairing in species of *Fritillaria* "with a normal distribution of chiasmata" is the result of the operation of a time limit which even in these species is too early to allow full association; he does not attribute them to an interruption by fixation of the process of zygotene pairing. Even if zygotene is completed, the possibility nevertheless remains for plants and especially such plants as *Fritillaria*, which reproduce largely by vegetative means, that the failure to pair completely may be due to structural hybridity. Inversions have been reported in nine species of this genus by Frankel (13, 14), Bennett (2), and Darlington (see 13). In *Triticum vulgare*, which is almost entirely self-fertilized and which reproduces only sexually, normal varieties show complete association at pachytene as Dark (3) has shown for a single nucleus of *Bellevallia*, while heterozygous deficiency mutations show unpaired loops at this stage (Huskins and Smith, manuscript in preparation) as McClintock (26) has shown in *Zea mays*. The complete pairing observed herein implies a total absence of structural hybridity in the Hymenoptera studied; this is presumably the result of the haploid constitution of the males; it follows that in the absence of the buffering effect of a second set of homologues selective elimination would come immediately into play (17).

That complete association of chromosomes at pachytene is the rule rather than the exception was the conclusion reached by many of the early workers on animals (cf. 46). Complete association of the chromosomes is found in other materials now being studied such as *Plethedon*, various Orthoptera, and man (material in the Department of Genetics, McGill University). It seems evident from McClintock's (26, 27) work on *Zea mays*, Levan's (see later) and Sax and Sax's (32) on *Allium*, and Ernst's (8, 9) on *Antirrhinum majus* that the same conclusion applies to plants. Hence the occurrence of unpaired regions appears to be the result of an interruption by fixation of the active process of pairing or of a lack of linear homology, rather than proof of the action of a time limit for pairing.

As was stated under the heading "Observations", in the 26 cells having incomplete pairing and in which the nucleolar chromosomes could be identified, these chromosomes were observed to be either completely or incompletely

** Since the foregoing was sent to press another species of sawfly, Gilpinia frutetorum, has been similarly analysed. Thirty-six nuclei at pachytene have been drawn and measured with results that agree in all respects with those obtained from the Neodiprion species.*

paired, while certain of the other chromosomes in the same cells were also either partially unpaired in the former or completely or partially paired in the latter. The nucleolar chromosomes were incompletely associated on eight occasions, the other chromosomes on 44. If incompletion of pairing is a random phenomenon the proportion expected is about 7.5 to 44.5—clearly the nucleolar chromosomes in these *Neodiprion* species are at no disadvantage during pairing relative to the other chromosomes. That they should be, if the association between homologues were initiated at zygotene, when the chromosomes are fine and drawn out, is a logical expectation. If, however, an active side by side association occurs by telophase of the last premeiotic division, as the writer believes, when no chromosome is handicapped in movement by an attached nucleolus, there should be no delay in the pairing of the nucleolar chromosomes.

Ernst (8) finds in *Antirrhinum majus* that at zygotene 88.5% of the cells have only one nucleolus. The nucleolar chromosomes, which are terminally attached to the nucleolus, were sometimes completely paired and sometimes separated on either side of the nucleolus. In the latter case they were seen to become united soon after leaving the nucleolus. In the remaining 11.5% the two nucleoli were in most cases relatively close to one another and the nucleolar chromosomes were associated in most of the proximal region. Whether in these rare cases the "static" nucleoli were actually hindering pairing or not is difficult to say because, if pairing is initiated centrically in *Antirrhinum*, as is probable, and progresses outwards, the unpaired regions may have been the result of fixing too early and thus interrupting the pairing process.

With regard to the third assumption, that the rapidity with which pachytene is completed is a function of chromosome length, the evidence appears strongly contrary.

Smith (34) has tabulated evidence from the literature showing that in most phyla of the Metazoa homologous chromosomes associate in pairs by the telophase of the last premeiotic mitosis. The observations of Stevens (38) and Foot and Strobell (12) on *Sagitta bipunctata* and *Allolobophora foetida* respectively leave no doubt that in these the pairing at telophase is intimate throughout the length of the chromosomes. Sutton (42), however, has demonstrated that while short chromosomes and short chromosome arms in the lubber grasshopper, *Brachystola magna*, associate completely at the last premeiotic anaphase, long chromosomes, many times the length of the former, associate only in their centric regions. Such an organism would be especially vulnerable to the action of a time limit for pachytene pairing.

In neither *Allolobophora* nor *Sagitta* are the chromosomes of the haploid complement of markedly different size (as they are in the orthopteran) being, in fact, somewhat similar in size range to those of the *Neodiprion* species studied here. The long chromosomes and the long chromosome arms in the lubber grasshopper are confined at telophase within compartments which open into a common chamber situated at the polar end of the nucleus. In these long chromosome arms association is therefore restricted to the centric

regions. The short chromosomes, however, are of insufficient length to allow their projection from the common chamber and hence they associate throughout their entire length. In other grasshoppers, with chromosomes of the same relative proportions, the side by side association of homologues, often initiated in the early gonial divisions (45), is completed by the end of the last premeiotic division.

Despite the fact that telophase pairing is localized at the centric ends of the chromosomes in *Brachystola*, pachytene association is nevertheless complete and chiasmata are formed apparently at random along the bivalents, although by diakinesis they are largely situated at or near the two ends of the bivalent. Foot and Strobell's (11, 12) photomicrographs of *Allolobophora* show that apparently complete pachytene pairing is similarly followed by chiasmata largely terminal at metaphase, even though in this case the premeiotic association was demonstrably complete. Unfortunately, Stevens' illustrations of the first metaphase chromosomes of *Sagitta bipunctata* fail to supply conclusive evidence of the relationship between the distribution of chiasmata and the complete pairing observed at telophase (38) and pachytene (39).

Pachytene pairing is not a simple continuation of telophase side by side association; this has to all intents and purposes been recognized by Fabergé (10). He points out that there is a difference between the initial pairing movements of leptotene–zygotene (the telophase association of the present author) and the close, or contact pairing of pachytene. He enumerates several points that he considers evidence against the general belief that the polarization of the ends is a necessary preliminary to normal pairing, for example, first, there is often no polarization, or it is variable in degree; second, in the bouquet the ends are often some distance apart; and, third, zipper pairing will not work in structural hybrids. Further, he considers the same objectinns apply to pairing being initiated at the centromere. It is clear that none of these objections is legitimate if side by side association arises at telophase, as has earlier been argued by the present author (34).

In the Diptera it has been conclusively proved that somatic pairing at prophase and metaphase is derived from the side by side association of homologues at the previous anaphase, and Hance (15) has shown that the prophase association is in the form of relational coiling. Similarly in the Diptera, it has been shown that the parallel association between homologues at the last premeiotic anaphase is supplanted in meiosis by relational coiling (29, 40, 41, 44, etc.). In other organisms this same relational coiling between chromosomes at meiosis replaces the relational coiling earlier apparent between chromatids at mitosis. Sparrow, Huskins, and Wilson (37) have given reasons for believing that somatic relational coiling is a carry-over from the previous division of the "plectonemic coiling" of the half chromatids. They deny that it arises as the result of an active twisting of the chromatids around one another as Darlington (4, 5) contends. Whether this is so or not, it is clearly recognized by Huskins (17) that the relational coiling *between chromo-*

somes seen at diplotene must result from an active twisting, and not from a carry-over. He considers it unproved that diplotene twists are the residuum after chiasma formation, pointing out that they might even be caused, not eliminated, by the chiasmata. However, the evidence available, meagre though it is, favours Darlington's interpretation, since relational coiling is demonstrably present both in the absence of crossing-over (male *Drosophila*) and in the absence of chiasma formation as shown by Richardson (31) on "asynaptic" *Crepis*; and Levan (24) in "asynaptic" triploid *Allium amplectens*.

From his extensive studies on the genus *Allium*, Levan has made valuable contributions to the knowledge of the relationships between pairing, relational coiling, and the position of chiasmata. Among the species that he has examined that supply evidence on these relationships are the eight listed in Table III.

TABLE III

THE RELATIONSHIPS BETWEEN PACHYTENE PAIRING, RELATIONAL COILING, AND CHIASMA POSITION IN *Allium*

Species	Diploid chromosome number	Degree of pachytene pairing	Extent of relational coiling	Distribution of chiasmata at metaphase	Reference
A. ammophylum	16	Complete	Complete	Random	Levan (23)
A. rosenbachianum	16	?	Complete	Random	Levan (23)
A. amplectens	28	Complete*	Complete	Random	Levan (24)
A. macranthum	28	Complete**	?	Random	Levan (22)
A. fistulosum	16	Complete	Complete	Localized	Levan (21)
A. farreri	16	Complete	Complete	Localized	Levan (23)
A. porrum	32	*Ca.* complete	Complete	Localized	Levan (25)
A. amplectens	21	Complete*	Complete	Zero	Levan (24)

* *Pairing complete except for exchanges of partner in multivalents.*
** *Pairing complete except for interlocking.*

From this table it will be seen that regardless of whether the chiasmata at metaphase are distributed at random, localized in regions close to the centromeres, or entirely absent, pachytene association is nevertheless complete or nearly so. Regarding the occasional unpaired portions, Levan points out that they result from (1) visible interlocking (*A. macranthum*), (2) exchanges of partner between the constituents of quadrivalents, or (3) structural hybridity as shown by dissimilarities in form and number of chromomeres (*A. amplectens*, 4*n*), and that at mid-pachytene they were not more frequently towards the ends of the bivalents (*A. porrum*).

Despite the subsequent variation in the position of chiasmata and also their absence in triploid *A. amplectens*, relational coiling was uniformly present

at diplotene[1]. It is thus evident that in *Allium* the position of chiasmata at metaphase bears no fixed relation to the extent to which pairing has proceeded at pachytene; the type of pairing at pachytene is not to be inferred from the distribution of chiasmata at metaphase. Bennett's (2) statement, "There is evidence, however, from the occasional persistence of relational coiling, at diakinesis, in arms of chromosomes having no chiasmata, that such arms have been unpaired or intermittently paired at pachytene", obviously ignores Levan's earlier evidence to the contrary.

Darlington (6) recognizes the fallacy of this assumption in that the chiasma frequency varies in different clones of *Fritillaria imperialis* despite their all having "nearly complete pairing". Clearly his precocity theory of meiosis and the principle of one-by-one attraction demand, first, that paired chromosomes are single at the time of pairing, and, second, that their division upsets this attraction; hence, he concludes, "torsion, or whatever else is responsible for their crossing-over potential" must vary. This Darlington considers to be the third prime variable of meiosis, the other two being the point of contact or the position in which pairing is initiated, and the time limit. It alone appears to be the variable the existence of which is positively established. Although it is almost certain that a random distribution of chiasmata at metaphase is a consequence of complete pairing at pachytene, it is doubtful whether any other position at metaphase bears any direct relation to the contact point and the postulated time limit, Mather (28) to the contrary. In *Drosophila melanogaster* a comparison of the genetic and cytological maps indicates that the chiasma frequency is lower in the centric regions than elsewhere. On the other hand, in those species of *Fritillaria* and *Mecostethus* having extreme centric localization, the chiasmata are concentrated around the centromere. Yet in all three, pairing starts in the neighbourhood of the centromere. Whatever it is that causes crossing-over must be responsible for this differential distribution, just as it must determine the different behaviour of the various *Allium* species.

In view of the conclusions reached independently by Fabergé (10) and Smith (34), one may expect that once the side by side association of homologues is established by telophase, the chromosomes will be in a position for the intimate pairing of the ensuing zygotene–pachytene to commence. Clearly, whether it will start from one or a number of points will depend on the degree to which telophase association had proceeded prior to demobilization at the resting stage. Possibly in some species showing restriction in chiasma distribution, such as *Fritillaria meleagris* and *Mecostethus grossus*, telophase association may be only partial and hence centrically localized; zygotene

[1] *In A. fistulosum and A. farreri, Levan attributed the more distal loops seen at diplotene to opening out between successive chiasmata. It seems evident now that these loops were the result of relational coiling, as Levan now interprets them in A. porrum. White (43), however, still considers them to be chiasmata freely distributed at diplotene, which have moved towards the centromere by metaphase, as reported by Hearne and Huskins (16) for Melanoplus femur-rubrum. Since chiasmata at diplotene are in unspiralized and somewhat widely spaced threads, which, however, become closely associated and spiralized by metaphase, it seems probable that the supposed movement is an optical effect due to decreasing parallax (18).*

pairing would then be hindered and, if prophase is of short duration, a time limit might come into play so that the unpaired regions divide and thereby interrupt pairing, as shown for *F. meleagris* by Huskins and Smith (19). In others, e.g., *Allium fistulosum*, etc., a similar delay might result in a lowering of the crossing-over potential (6) in distal regions but, if there were no interruption by division, pairing should be completed and relational coiling should be seen at diplotene. In yet others, such as "asynaptic" triploid *Allium*, the crossing-over potential might be reduced throughout the chromosomes below the necessary minimum. Terminal localization might then result not necessarily from distal contact but from the loss of crossing-over potential being restricted to the centric regions. Genetic data imply a variation in crossing-over potential in different regions of the chromosomes of *D. melanogaster*, yet it is in this species of all organisms that it is certain that association is intimate throughout the length of the chromosomes at somatic telophase and presumably at that of the last gonial division. It is therefore concluded that there is no fixed relationship between the intimacy of telophase association and pachytene pairing, the degree of diplotene relational coiling, and the distribution of metaphase chiasmata.

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STUDIES ON FACTORS INFLUENCING THE HEALTH OF PIGS

I. THE RELATIONSHIP OF BLOOD HAEMOGLOBIN CONCENTRATIONS TO RATE OF GAIN IN SUCKLING PIGS[1]

By W. E. Swales[2], E. W. Crampton[3], G. C. Ashton[4], and L. Choquette[5]

Abstract

The trends of blood haemoglobin concentrations in suckling pigs in a herd in which clinical anaemia does not occur have been studied and compared to rates of gain in body weight.

Ferrous sulphate and reduced iron were then used to keep the haemoglobin concentrations at higher levels, and statistical analysis of the observations showed a significant difference in body weight at weaning time between the treated and control groups.

The results indicate that pigs should be provided with a source of iron within a day or two after birth in order to ensure normal development and, possibly, resistance to disease.

Work on this and other possible factors in disease resistance is being continued.

Introduction

The importance of nutritional anaemia as a cause of loss in suckling pigs that are kept indoors has resulted in the collection by many workers of a large number of data on the nature, prevention, and treatment of the disease. In Canada a high percentage of litters are, of necessity, farrowed indoors and many of them have no access to soil and vegetation during the first few weeks of life. The precipitous drop in haemoglobin from the birth level, which approximates that of an adult animal, to one bordering on clinical anaemia occurs during the first 10 days of life. Unless this drop is checked by some form of therapy a serious disease often occurs, and it is now generally recognized that clinical anaemia predisposes to other pathological conditions. Hamilton, Hunt, and Carroll (4) define clinical anaemia in pigs as the condition that results when the blood haemoglobin concentration reaches a value of 3.5 gm. or less per 100 cc. of blood. As the haemoglobin level in the newborn pig approximates 11.5 gm.%, the intermediate stage between the birth and anaemia levels is relatively wide.

The work of Schofield (7) in Canada resulted in steps being taken to prevent clinical anaemia and only uninformed stock owners now fail to give attention to preventive measures for confined litters. However, many farmers do not

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supply any form of iron supplement until the pigs are several days old, at which time the haemoglobin may have dropped to less than 75% of the birth level. For this reason the effect on growth and resistance to disease of such subclinical anaemias is still open to question. According to Moe, Craft, and Thompson (6), groups of pigs that received various iron supplements and those that received no supplement but did not develop clinical anaemia, made similar gains in weight; however, the groups receiving iron were significantly heavier at weaning age than the untreated control group.

The herd of pigs that is used for research in animal nutrition at Macdonald College rarely suffers losses from anaemia. This has been ascribed to the dietary regime, which includes an abundance of iron (0.5% of ferric oxide in the supplement) in the ration of the pregnant and nursing sows and, theoretically, ensures an adequate storage in the new-born pig. In addition, the faeces of the sow has a high iron content and this, spread around the pens, is available for the suckling pigs. Because no clinical anaemia had occurred, no direct dosing of pigs had been practiced up to 1941. Although the records of growth of the majority of young pigs had been very satisfactory, an examination of the data for 1940 showed that a flattening of the growth curve between the ages of 10 and 21 days occurred. As this period appeared to coincide with a period of susceptibility to Ascaris infection in pigs in other parts of the Dominion (as found by Choquette (2)), in a preliminary survey, it was decided to investigate the blood haemoglobin levels in the litters born during 1941.

Procedure

Eight litters, comprising 61 pigs born during January, February, and March, 1941, were used for preliminary measurements of blood haemoglobin levels. The measurements were made by the Dare haemoglobinometer; one person took all the readings after it was determined by checking on a photoelectric colorimeter that his interpretations of the matching colours were relatively

TABLE I

OBSERVATIONS ON BLOOD HAEMOGLOBIN AND GAIN IN BODY WEIGHT OF 61 PIGS BORN IN JANUARY, 1941

Age (days)	Weight (lb.) and standard deviations	Weight (lb.)*	Daily gain (lb.)**	Haemoglobin (gm.%) and standard deviations
1	2.9 ± 0.3	2.8	—	9.9 ± 1.7
8	6.6 ± 0.9	6.8	0.50	6.7 ± 1.2
15	10.0 ± 1.3	10.1	0.47	5.7 ± 1.2
22	13.2 ± 1.9	12.9	0.41	5.9 ± 1.5
29	15.6 ± 2.4	15.6	0.39	6.5 ± 1.7
36	18.5 ± 2.9	18.4	0.40	7.6 ± 2.2
43	21.4 ± 3.1	21.6	0.45	8.5 ± 2.1
50	25.4 ± 4.1	25.3	0.53	10.5 ± 1.9

** A third degree curve fitted by the summation method of fitting polynomials (Fisher (3)).*

*** These values are the differences between the polynomials divided by the number of days between times of weighing.*

accurate. The results of these observations, together with data on the weights and rates of gain, are shown in Table I and Fig. 1. In Table I the standard deviations shown indicate the normal variation between individual pigs within litters, the difference between sows having been removed*.

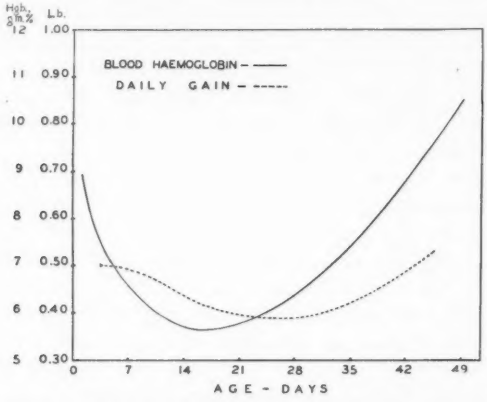

FIG. 1. *Blood haemoglobin and daily gain of 61 pigs born in January (Expt. I).*

The trend of the blood haemoglobin followed the expected curve (according to other workers, including Kernkamp (5), Moe, Craft, and Thompson (6)). The downward trend of the daily gain to the fourth week of life indicated a possible relationship of lower haemoglobin levels to growth.

Results

Tests were conducted with the next lot of litters that were born during May and June, 1941, in order to determine the effects of keeping the blood haemoglobin at higher concentrations. Two forms of iron were used for comparison of values; ferrous sulphate ($FeSO_4 . 2H_2O$) and iron reduced by hydrogen were chosen. The dose of ferrous sulphate was set at $4\frac{1}{2}$ grains (= 86 mg. iron) and that of reduced iron was set at 107 mg. Eight litters, comprising 75 pigs, were used, each litter being divided at random into three lots. One three grain and one one and one-half grain coated tablet of ferrous sulphate was given as the dose to each of one group, one capsule containing 107 mg. of reduced iron to each of the second group, and the third group was not dosed.** Bleeding for haemoglobin readings, administration of iron preparations and recording of body weights were first made on each litter between the 12th and 36th hour after birth and at weekly intervals thereafter to seven weeks.

* *Ashton, G. C. and Crampton, E. W. Rates of growth of bacon-type nursing pig. (Unpublished manuscript.)*

** *The iron preparations used for this experiment were supplied by the Veterinary Department of Ayerst, McKenna, and Harrison Ltd., Montreal.*

of age. The weekly data obtained were subjected to an analysis of variance according to the scheme shown on Table II.

TABLE II

ANALYSIS OF VARIANCE OF LIVE WEIGHTS OF TWO-DAY-OLD PIGS

Variance due to:	D.f.	Sums of squared deviations $S(x - \bar{x})^2$	Variance
All causes	74	21.68	
Between sows	7	5.35	0.76
Between treated groups	2	0.44	0.22
Between sexes	1	0.54	0.54
Remainder	64	15.36	0.24

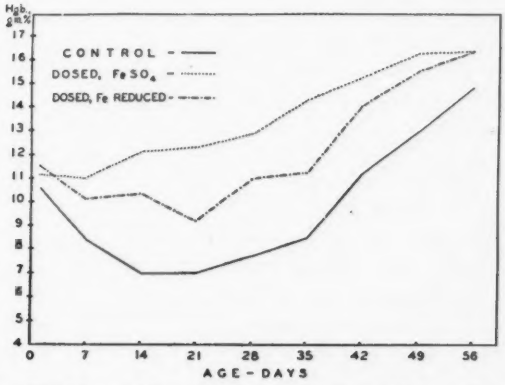

FIG. 2. *Blood haemoglobin of 75 pigs born in May and June (Expt. II).*

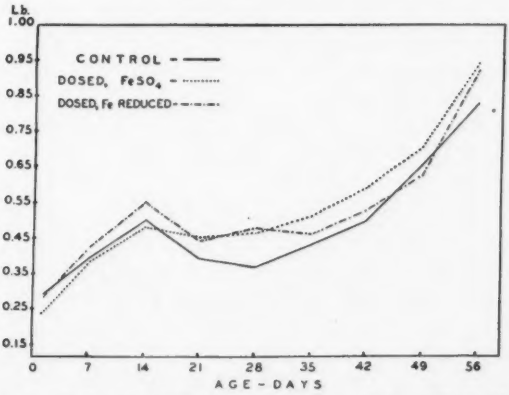

FIG. 3. *Daily gain of 75 pigs born in May and June (Expt. II).*

The mean weekly live weights and haemoglobin levels are shown in Table III, the latter also appearing as plotted curves in Fig. 2. The daily gains (as observed) are shown in Fig. 3.

TABLE III

MEAN WEEKLY LIVE WEIGHTS AND HAEMOGLOBIN LEVELS OF 75 PIGS BORN IN MAY AND JUNE, 1941, AND SUBJECTED TO IRON THERAPY

Treatment	Observations	Age (days)								
		1	7	14	21	28	35	42	49	56
Check	Haemoglobin, gm.%	10.7	8.4	7.0	7.0	7.8	8.5	11.2	13.0	14.9
	Weight (lb.)	3.2	5.4	8.8	11.6	15.2	17.2	20.7	25.3	30.7
Ferrous sulphate	Haemoglobin	11.2	11.0	12.1	12.3	12.9	14.3	15.3	16.3	16.4
	Weight	3.0	5.2	8.6	11.9	16.5	18.6	22.6	27.6	33.6
Reduced iron	Haemoglobin	11.6	10.1	10.4	9.2	11.0	11.3	14.1	15.6	16.4
	Weight	3.2	5.5	9.5	12.5	17.4	19.0	22.7	27.3	33.1

In estimating the results in terms of statistical analysis, Table IV was prepared because of the lack of definite differences at all stages of growth. Because the differences between groups were not statistically significant (at a probability (P) = 0.05) at all periods, the odds that the observed differences were due to treatment were calculated. In Table IV it will be noted that at 43, 50, and 56 days, the odds are 20 to 1 or better that the differences between the untreated and treated groups are real.

TABLE IV

EXPT. II. DIFFERENCES IN LIVE WEIGHTS OF PIGS AT SPECIFIED AGES

Age (days)	Check group vs. $FeSO_4$ group			Check group vs. Fe reduced group			$FeSO_4$ group vs. Fe reduced group		
	Differences		Observed P	Differences		Observed P	Differences		Observed P
	Obs.	Necessary (P=0.05)		Obs.	Necessary (P=0.05)		Obs.	Necessary (P=0.05)	
2	0.17	0.27	0.20	0.00	—	—	0.16	0.28	0.25
7	0.19	0.51	0.45	0.15	0.52	0.55	0.34	0.52	0.20
15	0.23	0.80	0.57	0.70	0.81	0.10	0.90	0.82	0.04
22	0.23	1.11	0.68	0.90	1.12	0.10	0.60	1.13	0.21
29	1.30	1.38	0.07	2.20	1.40	0.01	0.90	1.41	0.18
36	1.37	1.62	0.09	1.80	1.64	0.03	0.40	1.66	0.60
43	1.95	1.86	0.03	1.98	1.87	0.03	0.02	1.90	0.90
50	2.33	2.40	0.05	2.02	2.42	0.10	0.31	2.45	0.80
56	2.87	2.76	0.04	2.39	2.73	0.08	0.48	2.76	0.73

It is apparent that both forms of iron were effective in keeping the haemoglobin levels well above those of the undosed group, ferrous sulphate having shown a slight superiority in this respect. Both groups of dosed pigs appeared, to the casual observer, to be somewhat more thrifty and of a better colour than the control group. The difference between the average weaning weight of the group receiving ferrous sulphate and the control group was significant, as we have seen. The difference between the group receiving reduced iron and the control group closely approached the point of "significance"; there was no statistically significant difference between the treated groups (ferrous sulphate vs. reduced iron).

Discussion

The observations on both lots of pigs added support to the data of previous years that show a period of slower growth in suckling pigs between the first and fourth weeks of life. This flattening of the growth curve was modified by iron therapy in the experiment and thus it seems probable that a lowered concentration of blood haemoglobin, not reaching the point of clinical anaemia, has a direct effect upon gain in weight.

The possibility that cyclic periods of growth may be normal in pigs must not be overlooked, particularly in view of the data for other young animals as presented by Brody *et al.* (1). However, if such a phenomenon occurs, then the cyclic decrease in rate of gain in suckling pigs can be influenced by a dietary supplement that keeps the blood haemoglobin at a higher concentration.

The practical importance of the results lies in the finding of the significant increase in body weight at weaning time in the group that received the weekly doses of an iron salt. It is apparent that the thriftiness of the control group was adversely affected as a result of the drop in haemoglobin, even though no clinical symptom of anaemia appeared and no haemoglobin level suggesting clinical anaemia was found. If normal growth of suckling pigs is affected, then it is probable that resistance to infection by animal parasites and other organisms would be lowered.

The drop in haemoglobin during the first week of life is considerable and as this work has indicated the benefits of preventing this initial fall, measures of health protection should include the administration of some form of iron to pigs during the first day or two after birth. There is no mechanical difficulty in administering the tablets or capsules, as a new-born pig readily swallows even a No. 00 gelatine capsule with a sucking motion if it is placed on the tongue and the finger retained in the mouth for a few seconds.

It is now generally recognized that a pig is able to utilize various iron preparations to a wider extent than humans. The two forms generally used are the ones employed in the experiment; ferrous sulphate is low in cost and has a theoretical advantage in having traces of copper as an impurity, which acts as a catalyst in the utilization of iron. However, the commercial undried ferrous sulphate (copperas) contains only slightly more than 20% iron, whereas reduced iron contains 90 to 96% metallic iron; thus the greater bulk

is a factor for consideration. The many factors that influence the utilization of iron cannot be discussed here, but the dosages used are apparently safe and effective for the purpose. Overdosage must be avoided, as toxic effects are possible sequelae. For practical application it is probable that the doses of either preparation could safely be reduced in number to two, one soon after birth and one about 10 days later, as advised by Schofield.

Pigs may eat a small amount of soil very soon after birth, thus the practical method of providing soil fortified with an iron salt is not to be discouraged as a means of attaining the desired results.

Work on this and other possible factors in resistance to disease in suckling pigs is being continued.

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INDEX TO VOLUME 20

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